

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
12 September 2002 (12.09.2002)

PCT

(10) International Publication Number
WO 02/070502 A1

(51) International Patent Classification⁷: C07D 311/08,
311/16, 311/20, 311/58, A61K 31/353, A61P 35/00, 35/02

(74) Agents: HEISEY, Ross, Mitchell et al.; DAVIES COL-
LISON CAVE, Level 10, 10 Barrack Street, Sydney, New
South Wales 2000 (AU).

(21) International Application Number: PCT/AU02/00264

(22) International Filing Date: 8 March 2002 (08.03.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
PR 3633 8 March 2001 (08.03.2001) AU

(71) Applicant (for all designated States except US): NOVO-
GEN RESEARCH PTY LTD [AU/AU]; 140 Wicks Road,
North Ryde, New South Wales 2113 (AU).

(72) Inventors; and

(75) Inventors/Applicants (for US only): HEATON, Andrew
[AU/AU]; 2/46-48 Abbotsford Parade, Abbotsford, New
South Wales 2046 (AU). KELLY, Graham, Edmund
[AU/AU]; 47 Coolawin Street, Northbridge, New South
Wales 2063 (AU). KUMAR, Naresh [AU/AU]; 33 White
Avenue, Maroubra, New South Wales 2035 (AU).

(81) Designated States (national): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,
CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,
LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,
MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG,
SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ,
VN, YU, ZA, ZM, ZW.

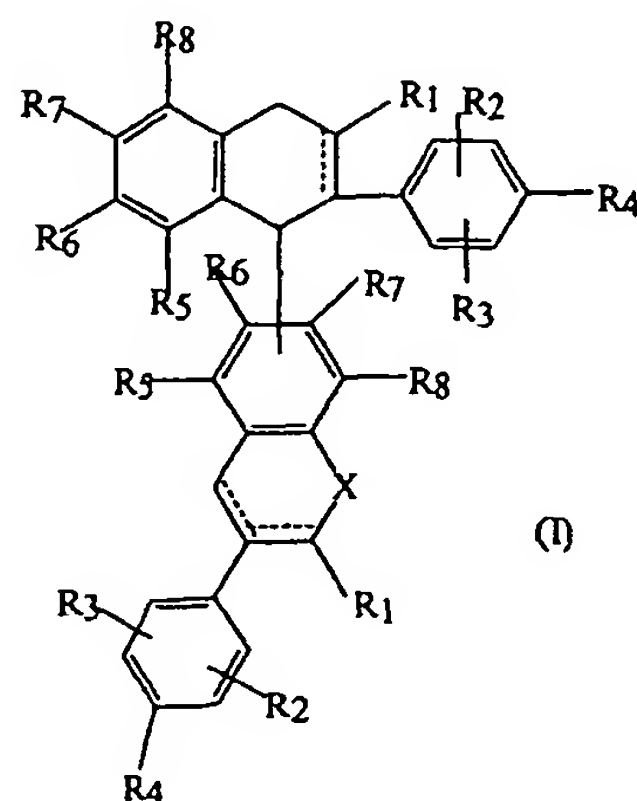
(84) Designated States (regional): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),
Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR,
GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent
(BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR,
NE, SN, TD, TG).

Published:

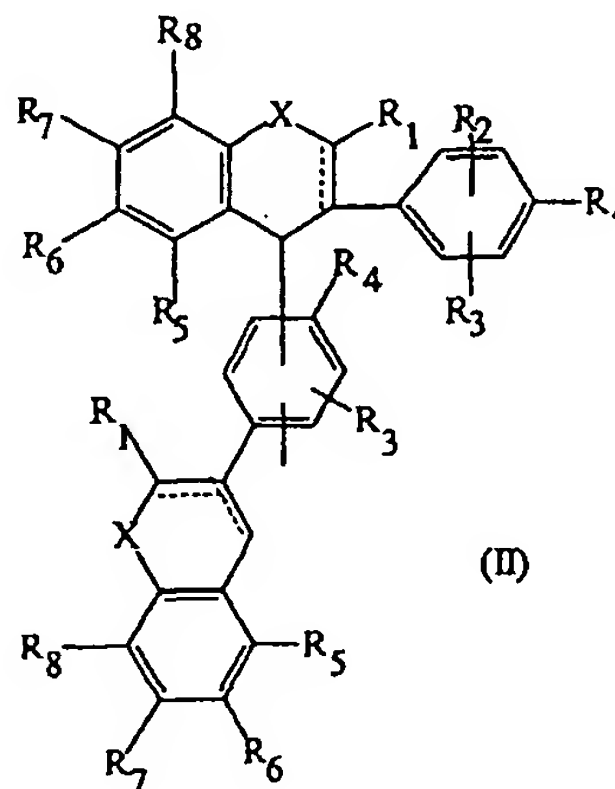
— with international search report

For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.

(54) Title: DIMERIC ISOFLAVONES



(I)



(II)

(57) Abstract: A compound of the general formula (I) or (II) wherein X is O, NR₄ or S and R₁-R₈ are as defined in the specification.

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DIMERIC ISOFLAVONES

5 **Field of the Invention**

The present invention generally relates to novel dimeric molecules based on the 1,2-diphenylpropane (isoflavonoid) ring structure. The present invention further relates to the synthesis of the dimeric isoflavonoid molecules, compositions containing the same and their use as therapeutic agents.

10

Background of the Invention

Naturally-occurring plant isoflavones are known to possess a wide range of fundamental biological effects on human cells including anti-oxidation and the up-regulation and down-regulation of a wide variety of enzymes and signal transduction mechanisms. Mitotic arrest
15 and cytotoxicity of human cancer cells, increased capillary permeability, increased cellular adhesion, increased response of vascular smooth muscle cells to vaso-relaxants, and agonism of estrogen receptors, are just a few examples of the responses of animal cells to the biological effects of naturally-occurring isoflavonoids.

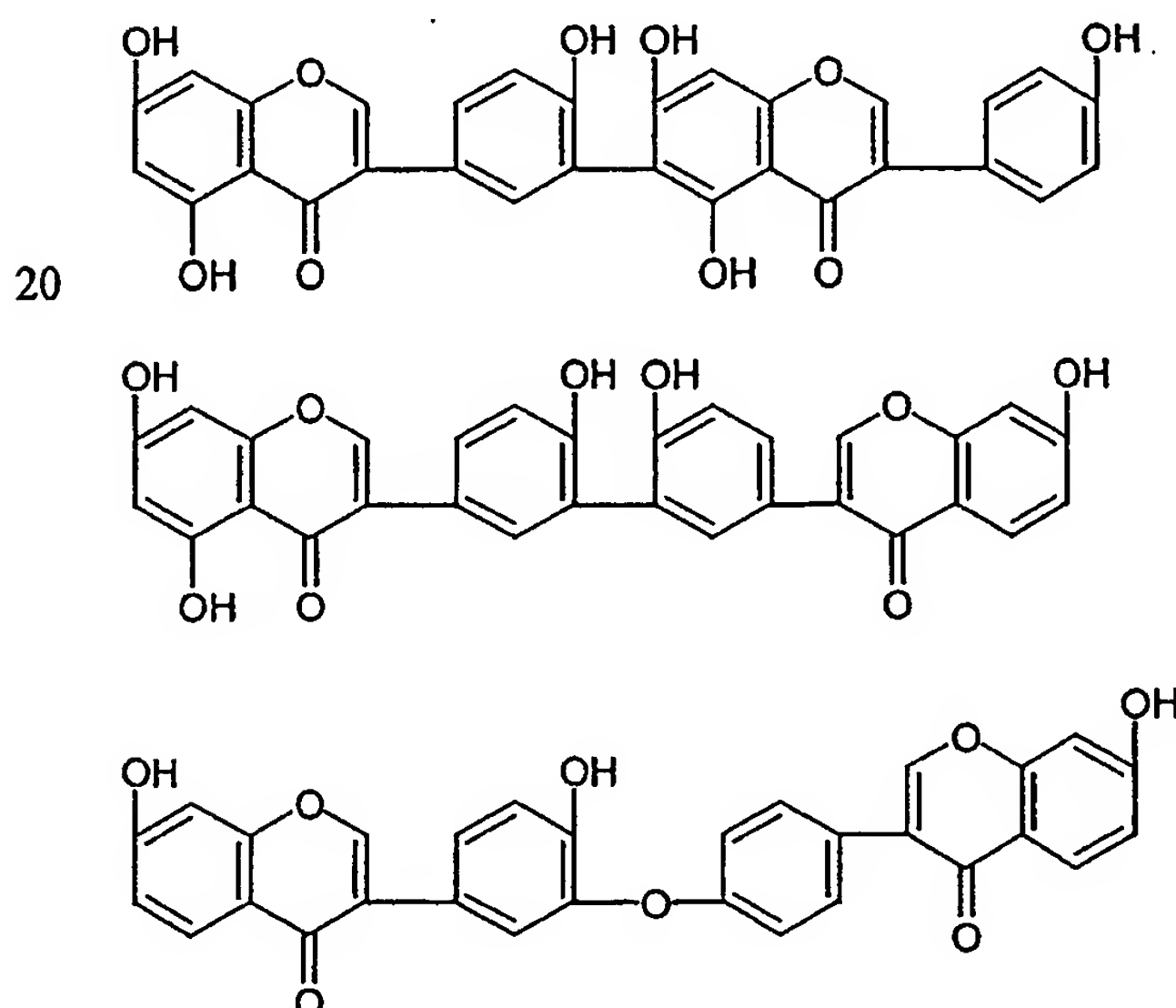
20 A range of therapeutic benefits as a result of these biological outcomes have been identified including the treatment and prevention of pre-menopausal symptoms such as pre-menstrual syndrome, endometriosis, uterine fibroids, hyperlipidaemia, cardiovascular disease, menopausal symptoms such as osteoporosis and senile dementia, alcoholism, benign prostatic hypertrophy, and cancers such as prostate, breast and large bowel
25 carcinomas [see WO 93/23069; WO 96/10341; US 5424331; JP 62-106017; JP 62-106016; US 5516528; JP 62-106016A2; JP 62-106017A2; JP 61-246124; WO 98/50026; WO 99/43335; WO 00/49009; WO 00/644438; WO 99/48496].

While over 700 different naturally occurring isoflavones are described, only a few are
30 confirmed as having potential therapeutic benefits in animals including humans. These include daidzein, genistein, formononetin, biochanin and glycitein. These and all naturally

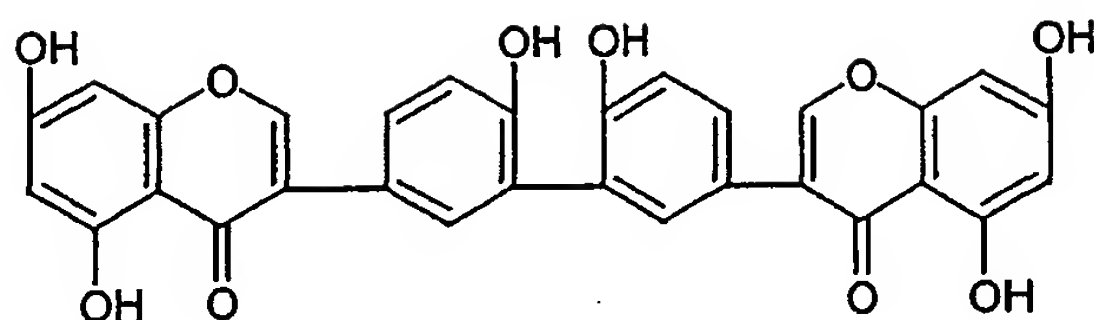
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occurring isoflavones are found in nature as the monomeric form either in a free state, or, more likely, bound to a carbohydrate moiety (glycoside). The isoflavone has to be separated from this moiety before it becomes biologically active.

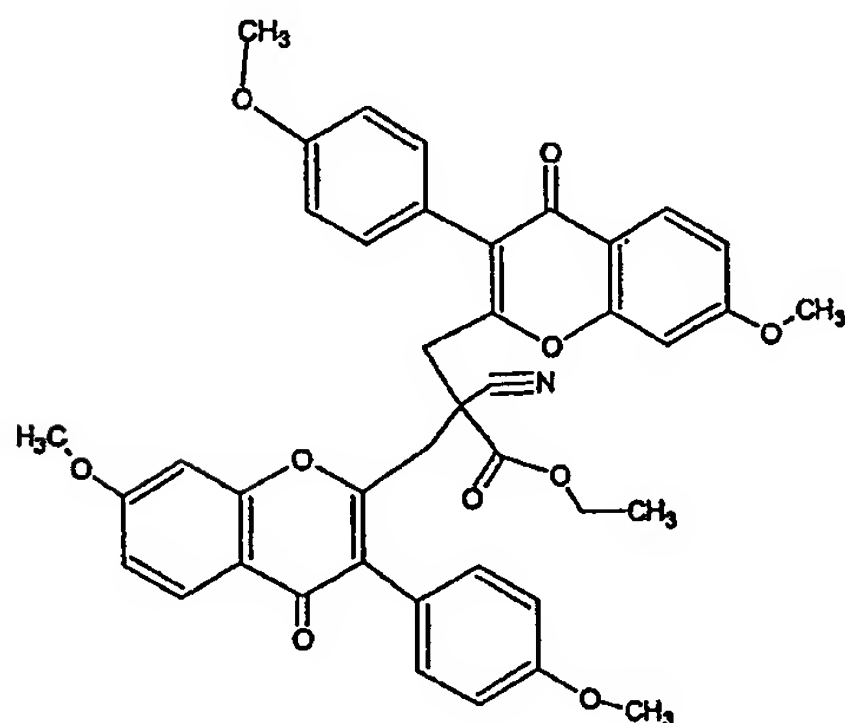
- 5 A number of compounds with a structure related to naturally occurring plant isoflavones are also described as having biological properties with potential therapeutic benefit to animals including humans. These include compounds that are naturally occurring metabolites of plant isoflavones produced by bacterial fermentation by gut flora and embrace compounds such as equol and *O*-desmethylangolensin [WO 93/23069; WO
10 98/08503; WO 01/17986; WO 00/66576]. Also included in this group is the synthetic isoflavonoid ipriflavone, which is developed for the treatment of postmenopausal osteoporosis [WO 91/14429] and a wide range of synthetic isoflavonoid analogues [WO 98/08503].
- 15 Recently, some interest has been expressed in the biological properties of dimeric forms of isoflavonoids. Four forms of bis-isoflavones with the following structures are claimed to have 5- α -reductase inhibitory activity useful for the treatment of prostatomegaly [JP 9067362-A].



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- 5 A C-C bridged bis-isoflavone dimer as shown below has also been described, but no biological activity or therapeutic benefit has been ascribed to it [Al-Maharik et al].



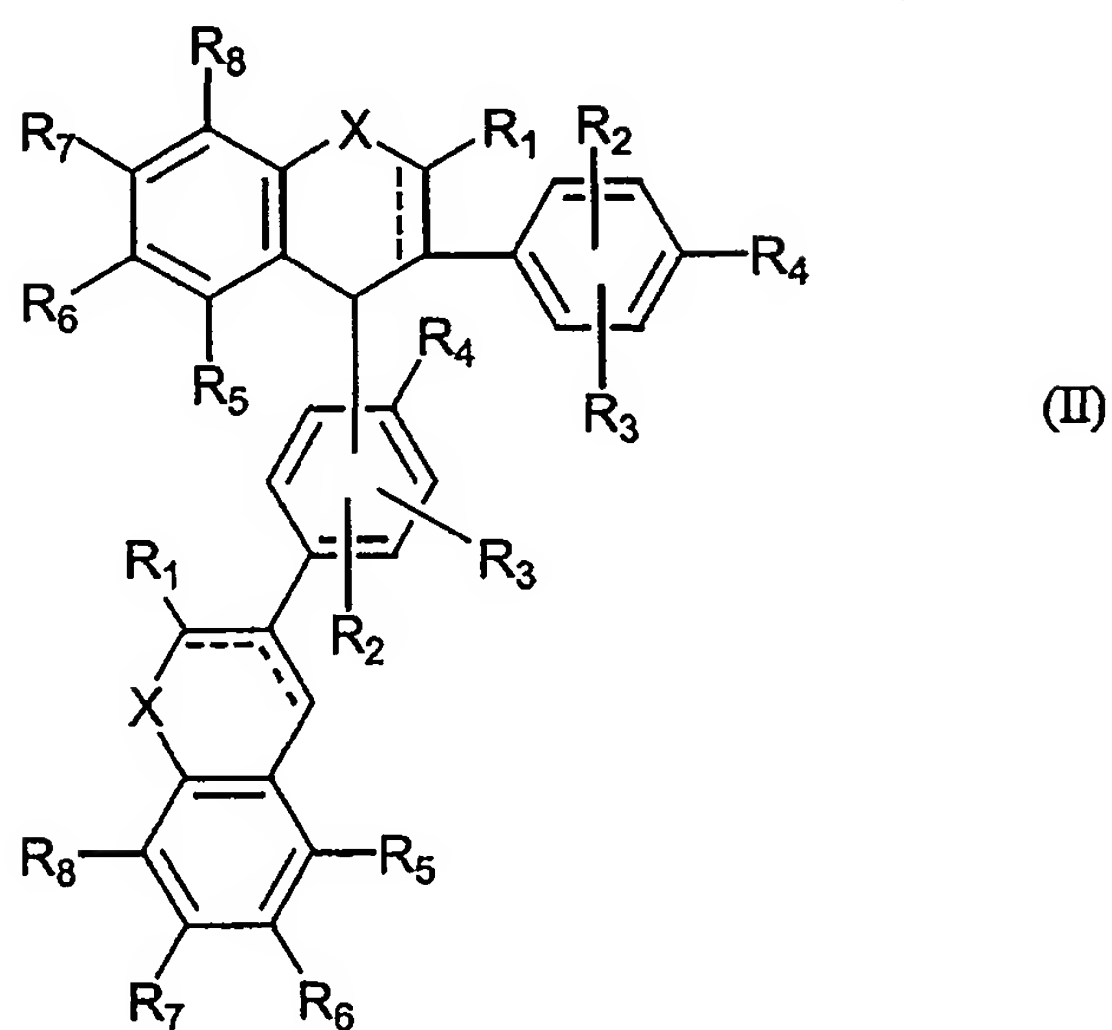
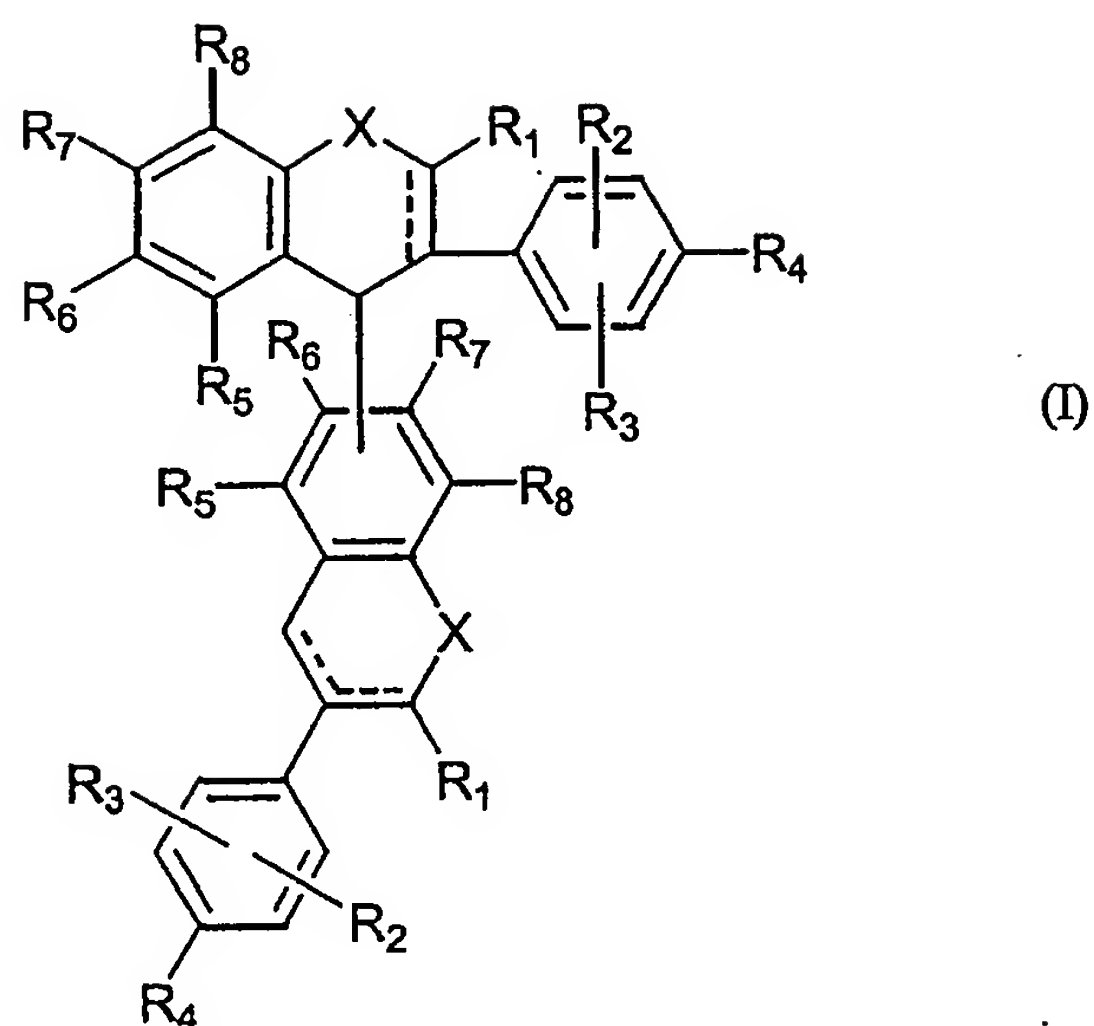
- 10 A requirement accordingly exists for new generation compounds that exhibit important pharmacological effects for use as prophylactics and in therapy.

Summary of the Invention

- Surprisingly, the present inventors have discovered a new class of molecules based on
 15 dimeric isoflavone compounds and derivatives. The dimeric molecules of the present invention generally show strong binding affinity for both estrogen receptors and hence exhibit remarkable physiological activity.

- Thus according to a first aspect of the present invention there is provided a compound of
 20 the general formulae (I) or (II):

- 4 -



5 wherein

R₁, R₂, R₃, R₄, R₅, R₆, R₇ and R₈ are independently hydrogen, hydroxy, OR₉, OC(O)H, OC(O)R₉, OS(O)R₉, OSi(R₁₀)₃, C(O)R₁₁, CO₂R₁₂, alkyl, haloalkyl, aryl, arylalkyl, thio, alkylthio, amino, alkylamino, dialkylamino, nitro or halo,

R₉ is alkyl, haloalkyl, aryl, arylalkyl or alkylaryl,

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R_{10} is independently hydrogen, alkyl or aryl,

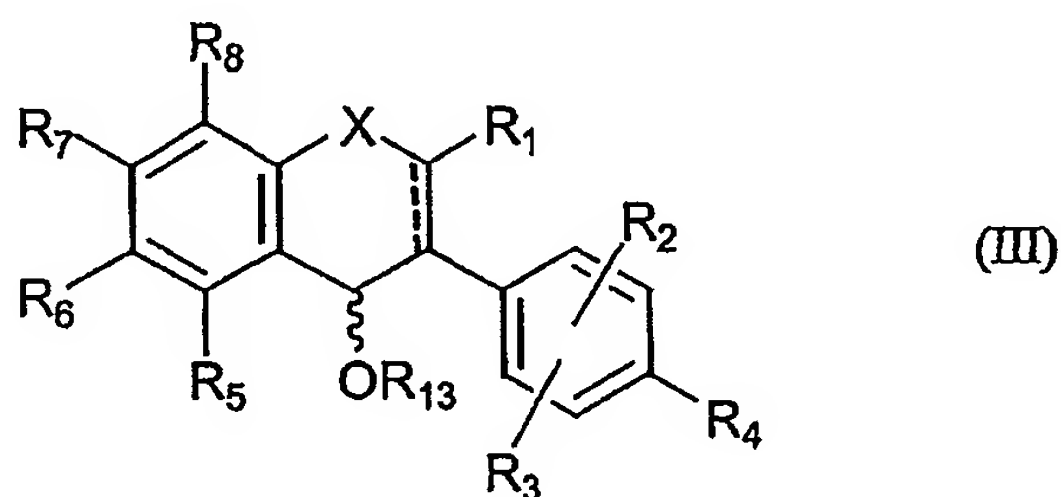
R_{11} is hydrogen, alkyl, aryl, arylalkyl or an amino acid, and

R_{12} is hydrogen, alkyl, haloalkyl, aryl or arylalkyl,

X is O, NR_4 or S, and

- 5 the drawing "—" represents either a single bond or a double bond,
which compounds include pharmaceutically acceptable salts thereof,
with the proviso that in formula (I) at least one of R_5 , R_6 , R_7 and R_8 is hydrogen.

- According to a second aspect of the present invention there is provided a process for the
10 preparation of a compound of formulae (I) or (II) by reacting a compound of the formula
(III)



- 15 wherein

R_1 , R_2 , R_3 , R_4 , R_5 , R_6 , R_7 and R_8 are independently hydrogen, hydroxy, OR_9 , $OC(O)H$, $OC(O)R_9$, $OS(O)R_9$, $OSi(R_{10})_3$, $C(O)R_{11}$, CO_2R_{12} , alkyl, haloalkyl, aryl, arylalkyl, thio, alkylthio, amino, alkylamino, dialkylamino, nitro or halo,

R_9 is alkyl, haloalkyl, aryl, arylalkyl or alkylaryl,

- 20 R_{10} is independently hydrogen, alkyl or aryl,

R_{11} is hydrogen, alkyl, aryl, arylalkyl or an amino acid, and

R_{12} is hydrogen, alkyl, haloalkyl, aryl or arylalkyl,

OR_{13} is hydroxy, another leaving group or an electron withdrawing group,

X is O, NR_4 or S, and

- 25 the drawing "—" represents either a single bond or a double bond,
which compounds include pharmaceutically acceptable salts thereof,

- 6 -

with the proviso that in the preparation of compounds of formula (I) at least one of R₅, R₆, R₇ and R₈ is hydrogen,
with a coupling agent.

5 It has surprisingly been found by the inventors that isoflavone dimers of the general formulae (I) and (II) have particular utility and effectiveness in the treatment, prophylaxis, amelioration defence against, and/or prevention of one of more of the following diseases and disorders (for convenience hereinafter referred to as the "therapeutic indications"):

(a) all forms of cancer (pre-malignant, benign and malignant) in all tissues of the body.

10 In this regard, the compounds may be used as the sole form of anti-cancer therapy or in combination with other forms of anti-cancer therapy including but not limited to radiotherapy and chemotherapy;

(b) diseases and disorders associated with inflammatory reactions of an abnormal or prolonged nature in any of the body's tissues including but not limited to
15 rheumatoid arthritis, tendonitis, inflammatory bowel disease, ulcerative colitis, Crohn's Disease, sclerosing cholangitis;

(c) papulonodular skin lesions including but not limited to sarcoidosis, angiosarcoma, Kaposi's sarcome, Fabry's Disease

(d) papulosquamous skin lesions including but not limited to psoriasis, Bowen's
20 Disease, and Reiter's Disease;

(e) actinic damage characterized by degenerative changes in the skin including but not limited to solar keratosis, photosensitivity diseases, and wrinkling;

(f) diseases and disorders associated with abnormal angiogenesis affecting any tissue within the body including but not limited to hemangiomas and telangiectasia;

25 (g) proliferative disorders of bone marrow including but not limited to megaloblastic disease, myelodysplastic syndromes, polycythemia vera, thrombocytosis and myelofibrosis;

(h) autoimmune disease characterized by abnormal immunological responses including but not limited to multiple sclerosis, Type 1 diabetes, systemic lupus erythematosus,
30 and biliary cirrhosis;

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- (i) neurodegenerative diseases and disorders characterized by degenerative changes in the structure of the neurological system including but not limited to Parkinson's Disease, Alzheimer's Disease, muscular dystrophy, Lou-Gehrig Disease, motoneurone disease;
- 5 (j) diseases and disorders associated with degenerative changes within the walls of blood vessels including but not limited to atherosclerosis, atheroma, coronary artery disease, stroke, myocardial infarction, hypertensive vascular disease, malignant hypertension, thromboangiitis obliterans, fibromuscular dysplasia;
- 10 (k) diseases and disorders associated with abnormal immunological responses including but limited to dermatomyositis and scleroderma;
- (l) diseases and disorders associated with degenerative changes within the eye including but not limited to cataracts, macular degeneration, retinal atrophy.

15 In particular the isoflavone dimers also surprisingly have been found to have a potent effect on the production and function of reproductive hormones such as estrogens and androgens. As a result of this, these compounds may be used in the treatment and prevention of one or more of the following disorders and diseases:

- 20 (a) conditions in women associated with abnormal estrogen/androgen balance including but not limited to cyclical mastalgia, acne, dysmenorrhoea, uterine fibroids, endometriosis, ovarian cysts, premenstrual syndrome, acute menopause symptoms, osteoporosis, senile dementia, infertility; and
- (b) conditions in men associated with abnormal estrogen/androgen balance including but not limited to benign prostatic hypertrophy, infertility, gynecomastia, alopecia hereditaria and various other forms of baldness.

25

Thus, according to a third aspect of the present invention there is provided a method for the treatment, prophylaxis or amelioration of a disease or disorder which method includes the step of administering a therapeutically effective amount of one or more compounds of formulae (I) and (II) to a subject.

30

According to a fourth aspect of the present invention there is provided the use of one or

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more compounds of formulae (I) and (II) in the manufacture of a medicament for the treatment of disease.

According to a fifth aspect of the present invention there is provided the use of one or more
5 compounds of formulae (I) and (II) as an anti-estrogen or selective estrogen receptor modulator (SERM).

According to a sixth aspect of the present invention there is provided an agent for the treatment, prophylaxis or amelioration of a disease which agent comprises one or more
10 compounds of formulae (I) or (II).

According to a seventh aspect of the present invention there is provided a pharmaceutical composition which comprises one or more compounds of formulae (I) and (II) in association with one or more pharmaceutical carriers and/or excipients.
15

According to a eighth aspect of the present invention there is provided a drink or food-stuff, which contains one or more compounds of formulae (I) and (II).

Throughout this specification and the claims which follow, unless the context requires
20 otherwise, the word "comprise", and variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

Detailed Description of the Invention

25 The dimeric molecules of the present invention are structurally related to monomeric isoflavone compounds and derivatives thereof. The term "isoflavone" as used herein is to be taken broadly to include ring-fused benzopyran molecules having a pendent phenyl group from the pyran ring based on a 1,2-diphenylpropane system. Thus, the classes of compounds generally referred to as isoflavones, isoflavenes, isoflavans, isoflavanones,
30 isoflavanols and the like are generically referred to herein as isoflavones, isoflavone derivatives or isoflavonoid molecules.

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The term "alkyl" is taken to mean both straight chain and branched chain alkyl groups such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, secbutyl, tertiary butyl, and the like. The alkyl group has 1 to 10 carbon atoms, preferably from 1 to 6 carbon atoms, more preferably methyl, ethyl propyl or isopropyl. The alkyl group may optionally be substituted by one or more of fluorine, chlorine, bromine, iodine, carboxyl, C₁-C₄-alkoxycarbonyl, C₁-C₄-alkylamino-carbonyl, di-(C₁-C₄-alkyl)-amino-carbonyl, hydroxyl, C₁-C₄-alkoxy, formyloxy, C₁-C₄-alkyl-carbonyloxy, C₁-C₄-alkylthio, C₃-C₆-cycloalkyl or phenyl.

10

The term "aryl" is taken to include phenyl and naphthyl and may be optionally substituted by one or more C₁-C₄-alkyl, hydroxy, C₁-C₄-alkoxy, carbonyl, C₁-C₄-alkoxycarbonyl, C₁-C₄-alkylcarbonyloxy or halo.

15 The term "halo" is taken to include fluoro, chloro, bromo and iodo, preferably fluoro and chloro, more preferably fluoro. Reference to for example "haloalkyl" includes monohalogenated, dihalogenated and up to perhalogenated alkyl groups. Preferred haloalkyl groups are trifluoromethyl and pentafluoroethyl.

20 The compounds of the invention include all salts, such as acid addition salts, anionic salts and zwitterionic salts, and in particular include pharmaceutically acceptable salts.

The term "pharmaceutically acceptable salt" refers to an organic or inorganic moiety that carries a charge and that can be administered in association with a pharmaceutical agent, for example, as a counter-cation or counter-anion in a salt. Pharmaceutically acceptable cations are known to those of skilled in the art, and include but are not limited to sodium, potassium, calcium, zinc and quaternary amine. Pharmaceutically acceptable anions are known to those of skill in the art, and include but are not limited to chloride, acetate, citrate, bicarbonate and carbonate.

30

Dimeric isoflavone molecules and derivatives have only recently appeared in the literature.

- 10 -

Japanese patent application No. JP 9067362-A (Sankyo Co. Ltd.) describes bis-isoflavones joined either by the pendent phenyl groups or by one pendent phenyl group of one monomer to the aromatic benzopyran ring of the other isoflavone monomer. The bis-isoflavones are thought to be useful as 5- α reductase inhibitors.

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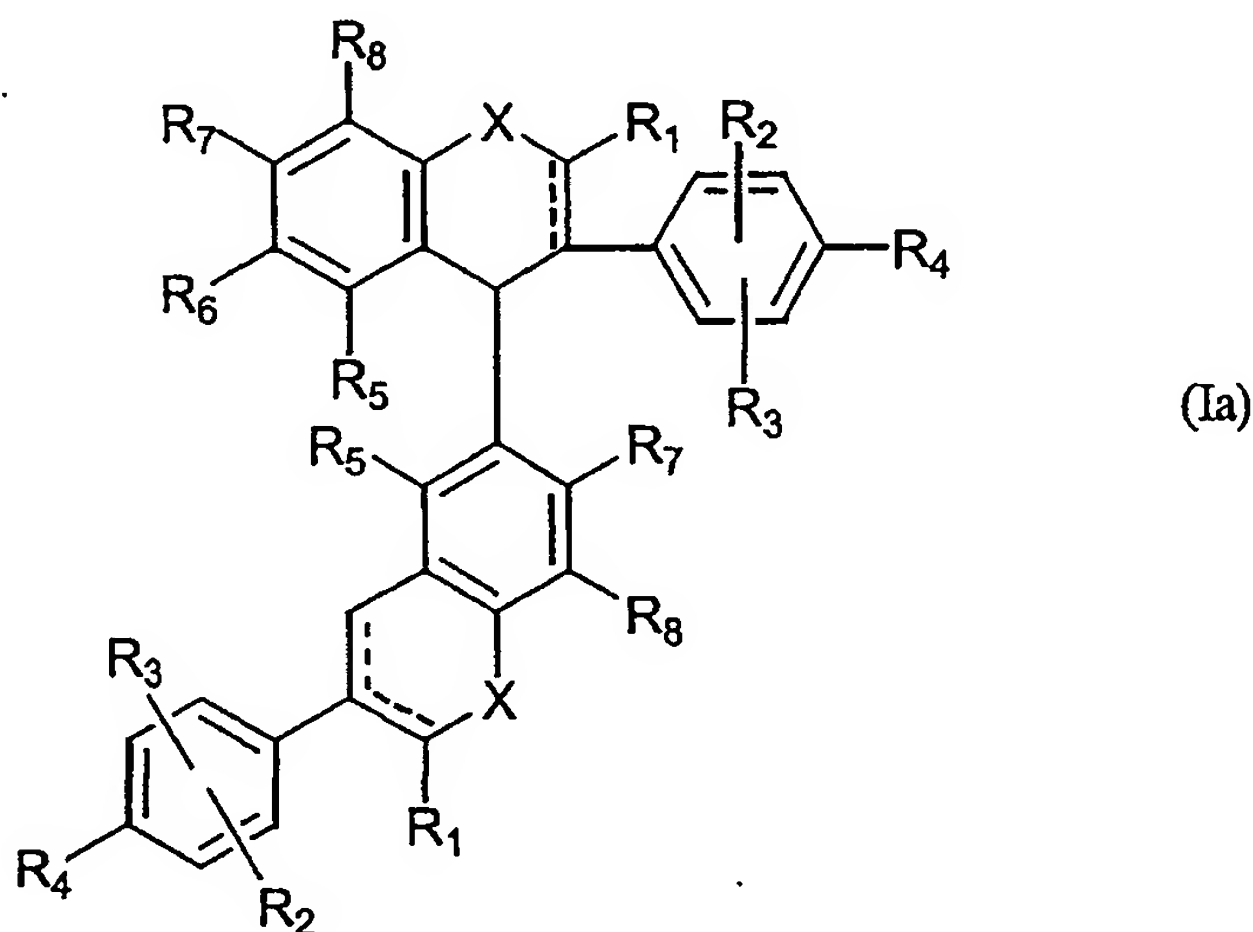
The synthesis of C-C bridged bis-isoflavones has also been described by the nucleophilic substitution of 2-bromomethyl isoflavone derivatives (Al-Maharik *et al.*). The double alkylation reaction gives rise to bridged bis-isoflavones attached through the 2-methyl groups. No activity has been ascribed to these newly synthesised bis-isoflavones.

10

In contrast, the dimeric molecules of the present invention are linked through the 4-position of the isoflavone pyran ring to either of the two aromatic rings. That is, the 4-position of the pyran ring is linked to the aromatic benzopyran ring (formula I), or to the pendent phenyl group (formula II).

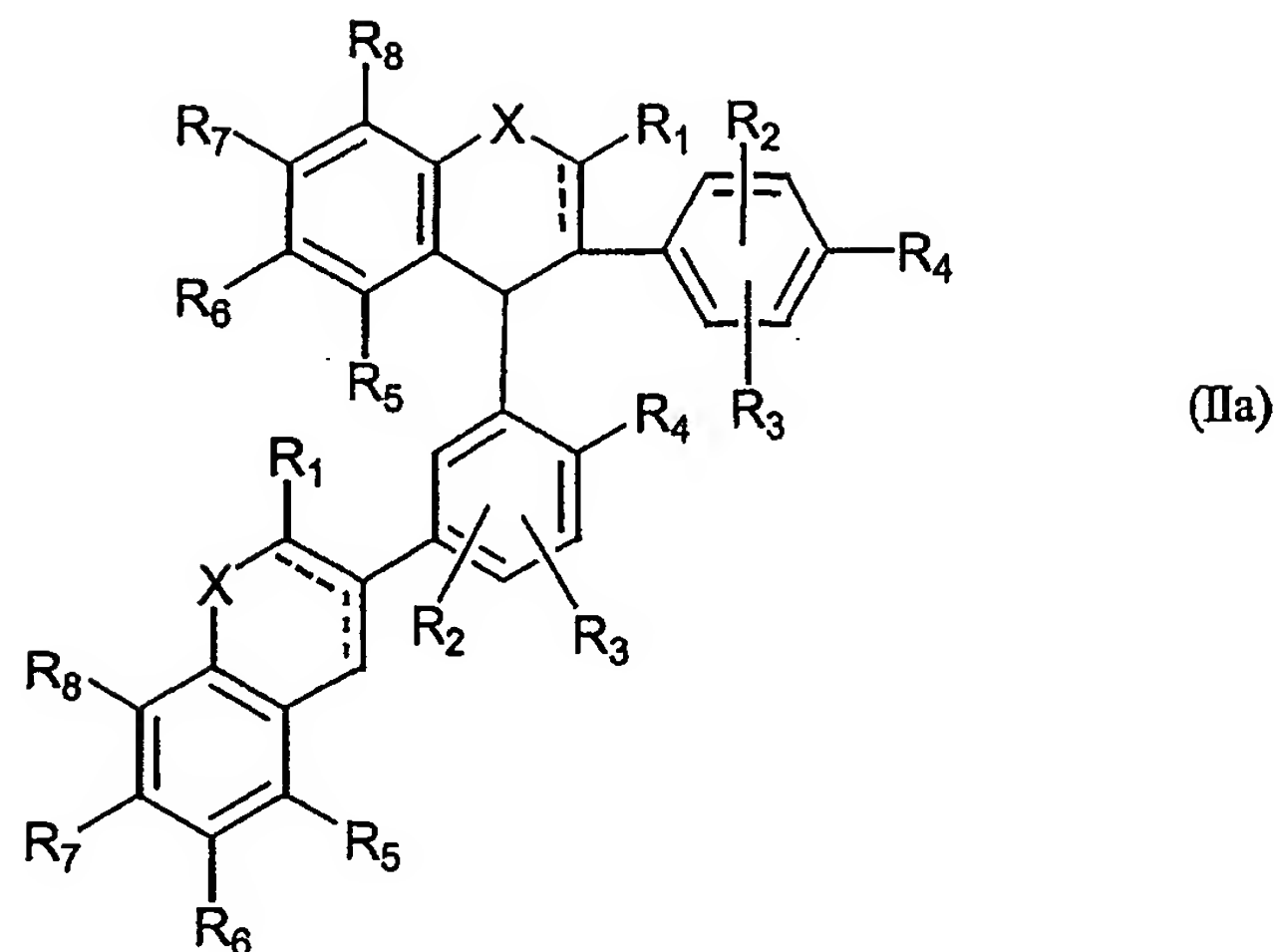
15

Preferred dimeric molecules of the present invention are depicted by the general formulae (Ia) and (IIa):



20

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wherein

- R₁, R₂, R₃, R₄, R₅, R₆, R₇ and R₈ are independently hydrogen, hydroxy, OR₉, OC(O)H,
 5 OC(O)R₉, OS(O)R₉, OSi(R₁₀)₃, C(O)R₁₁, CO₂R₁₂, alkyl, haloalkyl, aryl, arylalkyl,
 thio, alkylthio, amino, alkylamino, dialkylamino, nitro or halo,
 R₉ is alkyl, haloalkyl, aryl, arylalkyl or alkylaryl,
 R₁₀ is independently hydrogen, alkyl or aryl,
 R₁₁ is hydrogen alkyl, aryl, arylalkyl or an amino acid, and
 10 R₁₂ is hydrogen, alkyl, haloalkyl, aryl or arylalkyl,
 X is O, NR₄ or S, and

the drawing "----" represents either a single bond or a double bond,

which compounds include pharmaceutically acceptable salts thereof,

with the proviso that in Formula (Ia) at least one of R₅, R₆, R₇ and R₈ is hydrogen;

15

more preferably they have the following substituents wherein

R₁, R₂, R₃, R₄, R₅, R₇ and R₈ are independently hydrogen, hydroxy, OR₉, OC(O)R₉,
 OS(O)R₉, alkyl, aryl, arylalkyl, thio, alkylthio, bromo, chloro or fluoro,
 R₆ is hydrogen,

20 R₉ is alkyl, fluoroalkyl or arylalkyl, and

X is O;

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more preferably they have the following substituents wherein

R₁ and R₆ are hydrogen,

R₂, R₃, R₅ and R₈ are independently hydrogen, hydroxy, OR₉, OC(O)R₉, alkyl, aryl or
5 arylalkyl,

R₄ and R₇ are independently hydroxy, OR₉ or OC(O)R₉,

R₉ is methyl, ethyl, propyl, isopropyl or trifluoromethyl, and

X is O; and

10 most preferably they have the following substituents wherein

R₁ and R₆ are hydrogen,

R₂, R₃, R₅ and R₈ are independently hydrogen, hydroxy, OR₉, OC(O)R₉ or methyl,

R₄ and R₇ are independently hydroxy, OR₉ or OC(O)R₉,

R₉ is methyl, and

15 X is O.

Most preferably the novel dimeric compounds of formulae (I) and (II) are:

Tetraacetoxy 6-(4-(isoflavan-4',7-diol))-isoflav-3-ene-4',7-diol (2)

6-(4-(Isoflavan-4',7-diol))-isoflav-3-ene-4',7-diol (3)

20 Tetraacetoxy 6-(4-(isoflavan-4',7-diol))-isoflavan-4',7-diol (4)

6-(4-(Isoflavan-4',7-diol))-isoflavan-4',7-diol (5)

6-(4-(4',7-Dimethoxyisoflavan))-4',7-dimethoxyisoflav-3-ene (6)

6-(4-(4',7-Dimethoxyisoflavan))-4',7-dimethoxyisoflavan (7)

25 Tetraacetoxy 3'-(4-(isoflavan-4',7-diol))-isoflav-3-ene-4',7-diol (8)

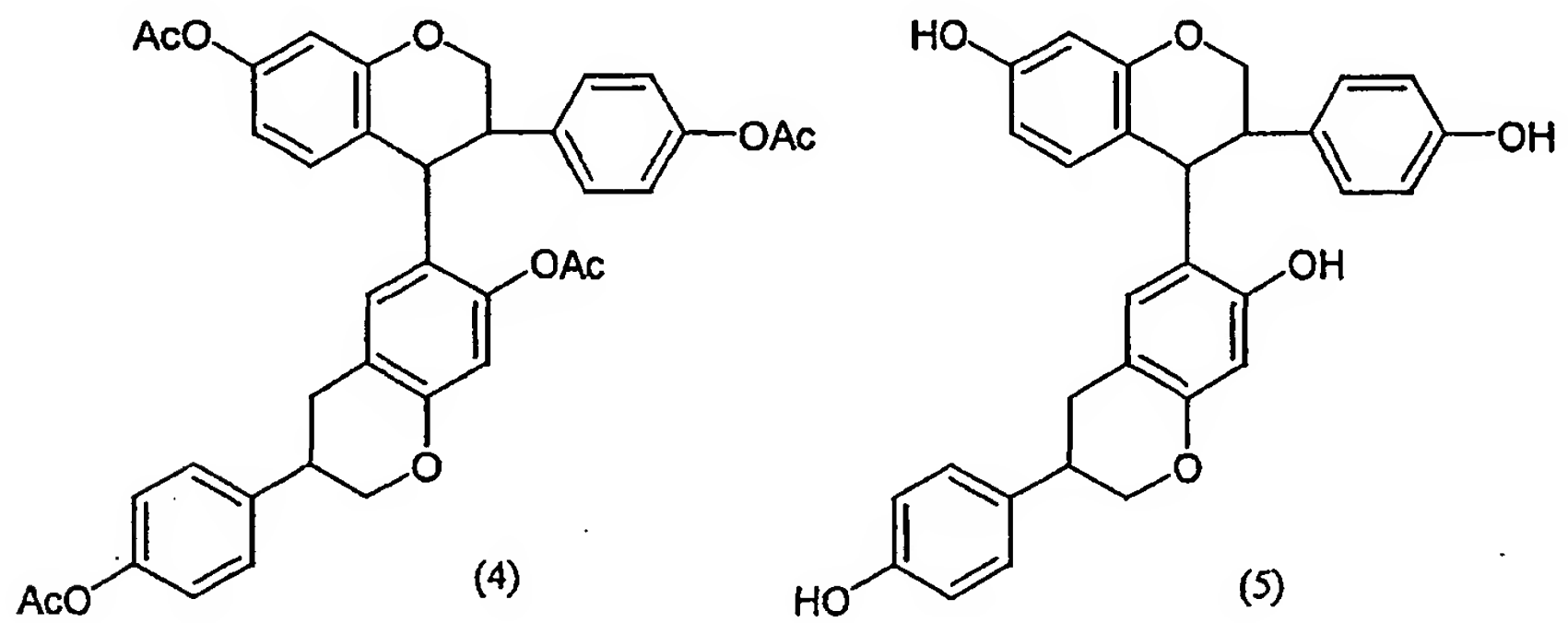
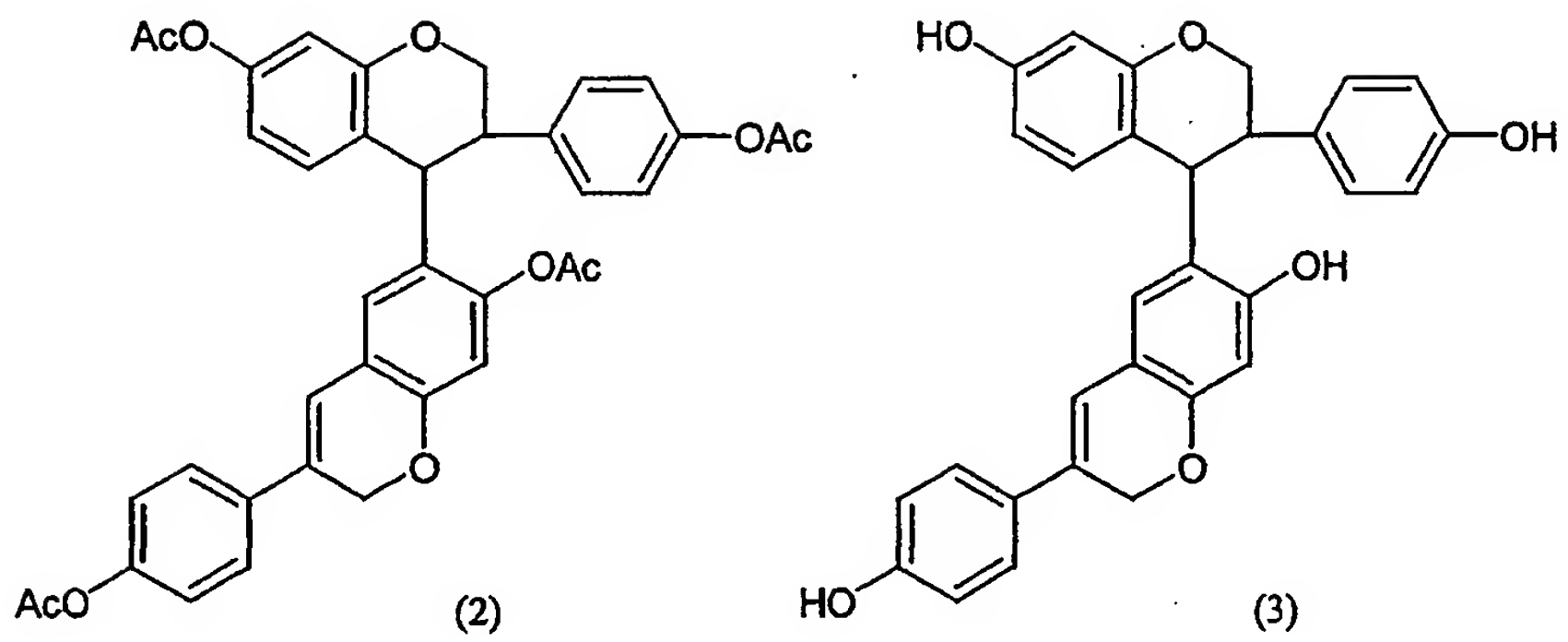
3'-(4-(Isoflavan-4',7-diol))-isoflav-3-ene-4',7-diol (9)

Tetraacetoxy 3'-(4-(isoflavan-4',7-diol))-isoflavan-4',7-diol (10)

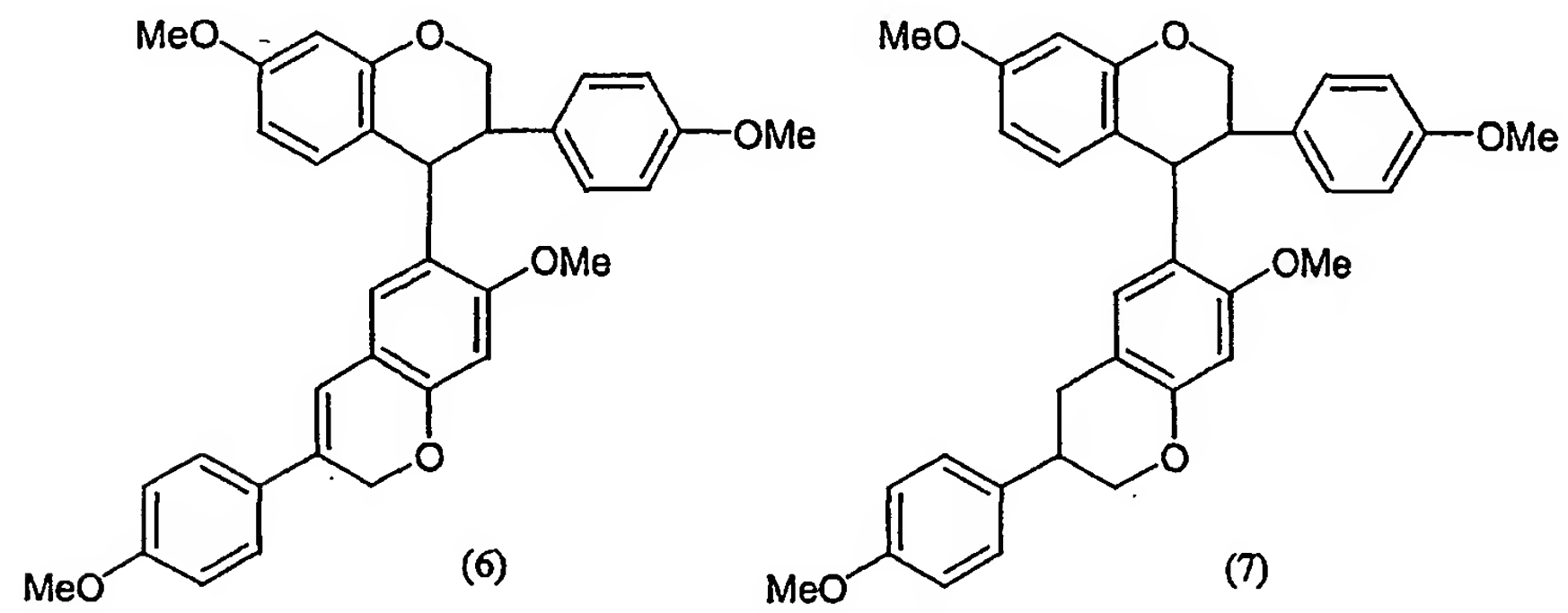
3'-(4-(Isoflavan-4',7-diol))-isoflavan-4',7-diol (11)

30

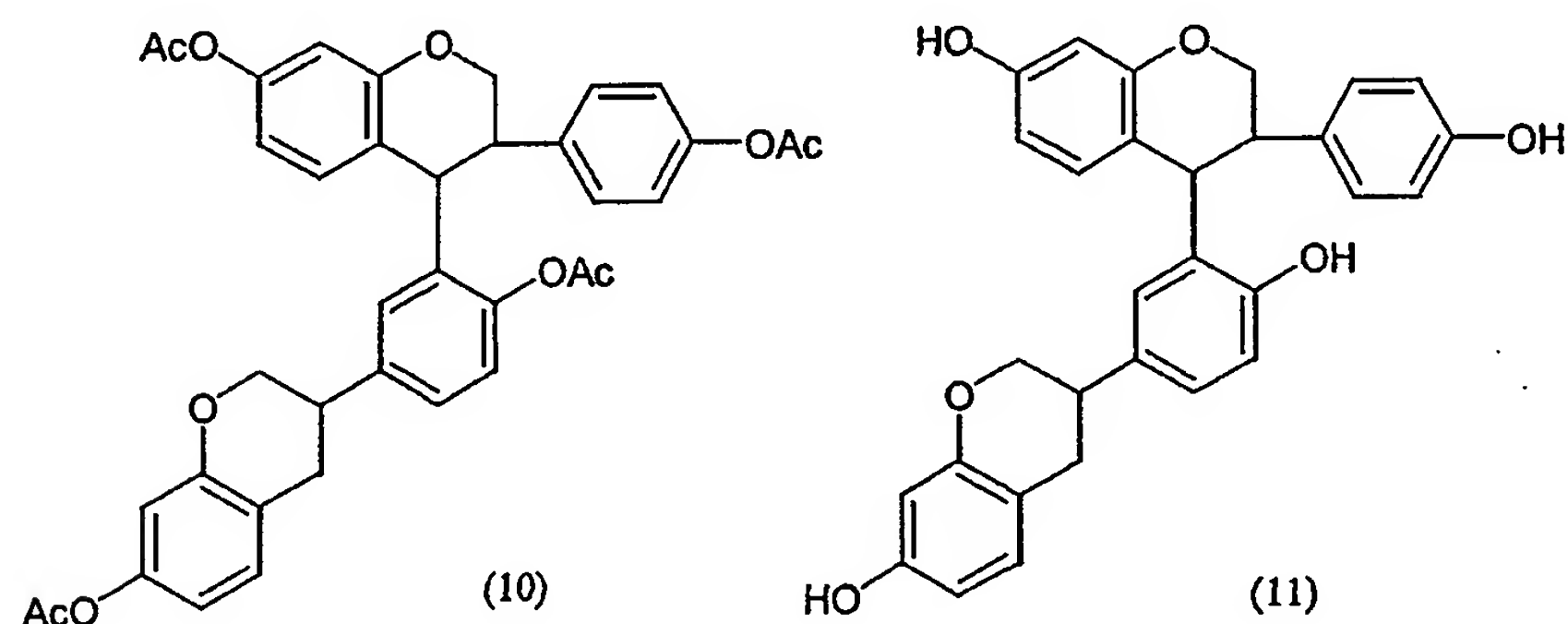
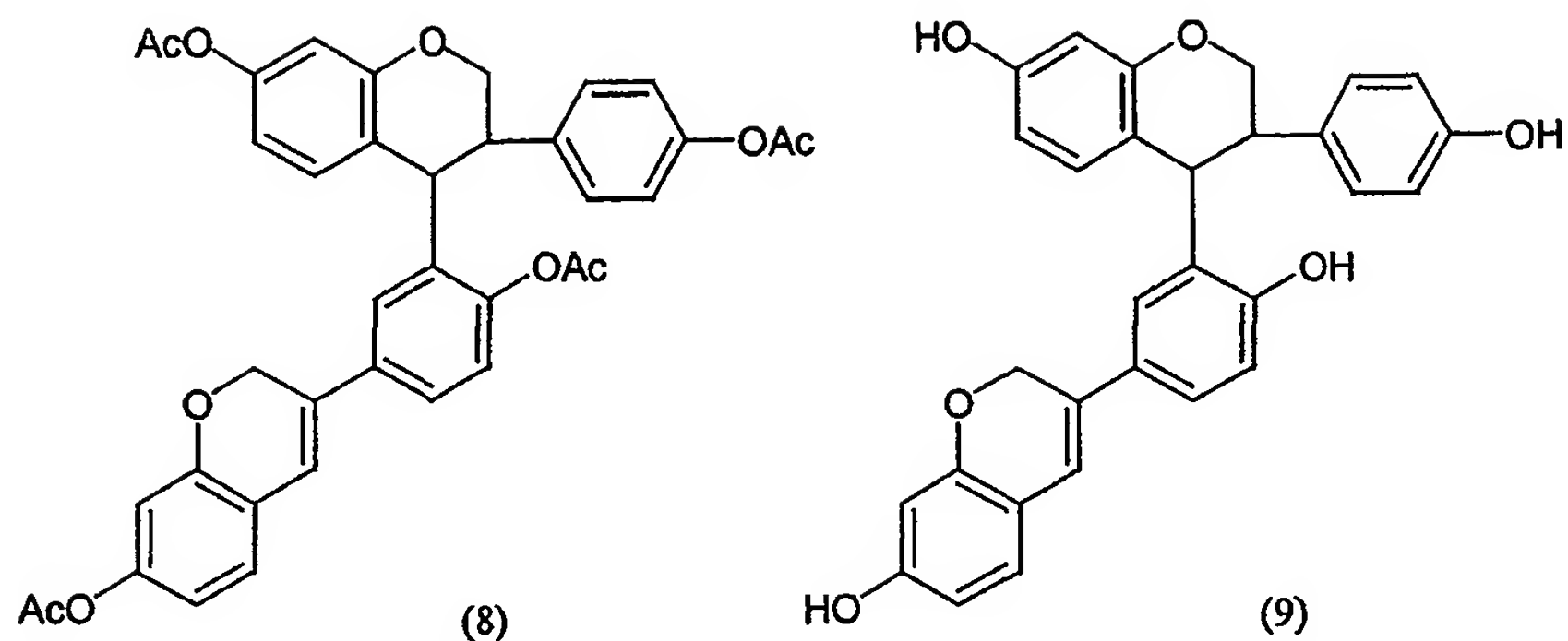
Compounds 2-11 are depicted by the following structural formulae:



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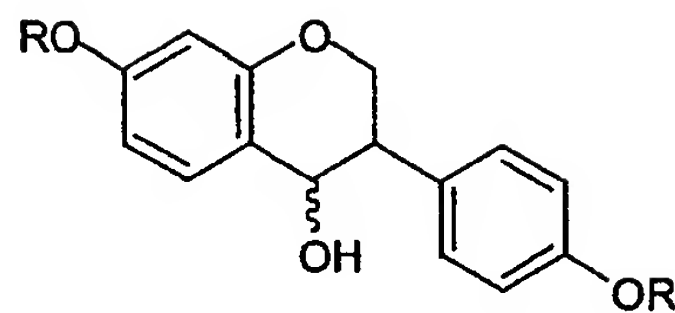
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Without wishing to be limited to theory, it is believed that the dimeric molecules of the present invention are formed as a result of an electrophilic aromatic substitution of one of the aromatic rings. Generally, plant-based isoflavone compounds have hydroxy groups in the 4'- and 7-positions, at least, on the isoflavone skeleton. These electron donating groups are thought to assist the electrophilic aromatic substitution and to direct substitution *ortho* or *para* to the electron donating substituent.

In a particularly preferred embodiment of the invention, the isoflavone diacetoxo tetrahydrodiadzein (1), R=Ac, is coupled to form a dimeric molecule upon dehydration of the monomer with phosphorus pentoxide.

15

- 15 -



(1)

Electrophilic substitution of the ring-fused benzene ring of (1) occurs preferentially alpha to the 7-oxy substituent and beta to the ring junction. The beta position is thought to be more available due to smaller steric constraints than that of the position alpha to the ring junction. The pyran oxygen is thought to assist in the selectivity of substitution by activation of the ring fused benzene to electrophilic aromatic substitution, in preference to substitution of the pendent phenyl group.

Other synthetic methods well known to those skilled in the art may also be employed in the synthesis of the dimeric molecules of the present invention. Such other suitable synthetic methods include Friedel-Crafts alkylation coupling of two isoflavones or derivatives thereof. Typical Friedel-Crafts reaction conditions are used with reagents such as aluminium chloride, boron trifluoride or similar Lewis acid catalysts. The coupling reaction is not limited to Friedel-Crafts type conditions, but may include other reaction conditions that can generate a "carbocation" species such as the conversion of a secondary alcohol to a phosphinic ester or similar facile leaving group. Oxidative coupling reaction conditions such as hydrogen peroxide, dilute permanganate, phosphorous pentoxide or other oxidative compounds may also be employed in the synthesis of the dimeric molecules of the invention.

Linking two monomeric isoflavone compounds or derivatives with a bridging group may also lead to dimeric isoflavone molecules of the present invention. Suitable divalent bridging groups include, for example, -O-, -S-, -CH₂-, -(C(R)H)_n-, -NR-, or -C=O.

Chemical functional group protection, deprotection, synthons and other techniques known to those skilled in the art may be used where appropriate to aid in the synthesis of the

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compounds of the present invention, and their starting materials.

The preferred starting isoflavone monomer, diacetoxy tetrahydrodaidzein (1) R=Ac, may be obtained by standard procedures known in the art. Particular reference is made to
5 published International patent applications WO 98/08503 and WO 00/49009, and references cited therein, (Novogen Research Pty Ltd) for useful synthetic methods for the production of (1) and related isoflavones.

However, the isoflavone monomers for use in the present invention may be derived from
10 any number of sources readily identifiable to a person skilled in the art. By way of example, the isoflavones may be purchased commercially or extracted from plant sources. Those skilled in the art will readily be able to identify suitable plant species which may be used to derive suitable isoflavone extracts for use in the present invention, however, plants of particular use in the invention include chickpea, soy and clover species, for example.
15 More preferably, an isoflavone extract is obtained from red clover or subterranean clover species.

An isoflavone extract may be prepared by any number of techniques known in the art. For example, suitable isoflavone extracts may be prepared by water/organic solvent extraction
20 from the native plant source. It will be appreciated that an isoflavone extract may be prepared from any single tissue of a single species of plant or a combination of two or more different tissues thereof. Similarly, an extract may be prepared from a starting material which contains a heterogeneous mixture of tissues from two or more different species of plant.

25

Generally, where an isoflavone extract is prepared from plant material, the material may be comminuted or chopped into smaller pieces, partially comminuted or chopped into smaller pieces and contacted with water and an organic solvent, such as a water miscible organic solvent. Alternatively, the plant material is contacted with water and an organic solvent
30 without any pre-treatment. The ratio of water to organic solvent may be generally in the range of 1:10 to 10:1 and may, for example, comprise equal proportions of water and

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solvent, or from 1% to 30% (v/v) organic solvent. Any organic solvent or a mixture of such solvents may be used. The organic solvent may preferably be a C2-10, more preferably a C1-4 organic solvent (such as methanol, chloroform, ethanol, propanol, propylene glycol, erythrite, butanol, butanediol, acetonitrile, ethylene glycol, ethyl acetate, glycidol, glycerol dihydroxyacetone or acetone). Optionally the water/organic solvent mixture may include an enzyme which cleaves isoflavone glycosides to the aglycone form. The mixture may be vigorously agitated so as to form an emulsion. The temperature of the mix may range, for example, from an ambient temperature to boiling temperature. Exposure time may be between one hour to several weeks. One convenient extraction period is twenty-four hours at 90°C. The extract may be separated from undissolved plant material and the organic solvent removed, such as by distillation, rotary evaporation, or other standard procedures for solvent removal. The resultant extract containing water soluble and non-water soluble components may be dried to give an isoflavone-containing extract, which may be formulated with one or more pharmaceutically acceptable carriers, excipients and/or auxiliaries according to the invention.

An extract made according to the description provided in the previous paragraphs may contain small amounts of oil which include isoflavones in their aglycone form (referred to herein as isoflavones). This isoflavone enriched oil, may be subject to HPLC to adjust the isoflavone ratios, or, if it is at the desired isoflavone ratio, may be dried, for example in the presence of silica, and be formulated with one or more carriers, excipients and/or auxiliaries to give an isoflavone containing extract. Alternatively, the isoflavones contained in said small amounts of oil may be further concentrated by addition to the oil of a non-water soluble organic solvent such as hexane, heptane, octane acetone or a mixture of one or more of such solvents. One example is 80% hexane, 20% acetone w/w having high solubility for oils but low solubility for isoflavones. The oil readily partitions into the organic solvent, and an enriched isoflavone containing extract falls out of solution. The recovered extract may be dried, for example in an oven at 50°C to about 120°C, and formulated with one or more pharmaceutically acceptable carriers, excipients and/or auxiliaries.

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Other suitable methods may be found in, for example, Chang *et al* which discloses methods appropriate for the synthesis of various isoflavones and derivatives thereof.

5 The dimeric isoflavone structures depicted in formulae (Ia) and (IIa) possess an inherent ability to restrict the conformation between the two monomeric structures to an orthogonal relationship. It is hypothesised that the apparent greater conformational rigidity of the orthogonal relationship and the inherent ability to define a displacement of H bond donors in the ligand binding domain monomeric section of the dimer leads to moderation of the normal agonistic actions of the monomer for cellular receptors. In most cases that
10 moderation results in up-regulation of the biological activity, while in some cases it results in down-regulation of that activity. That is, the biological profile of the monomer which to a large extent is predictable based on the known structure-function relationship established from the experience with screening large libraries of such compounds, is unpredictable with the dimeric forms. Functions which are moderated with the conversion from the
15 monomeric to the dimeric forms are as follows: absolute levels of agonism and antagonism of hER- α and hER- β as well as the relative levels of agonism/antagonism of both receptor types; inhibition of 5- α - reductase and 17 β -steroidaldehydrogenase; anti-oxidation; mitotic arrest and induction of apoptosis of cancer cells; prostaglandin and inflammatory cytokine release; vasoactivity; high density lipoprotein and low density lipoprotein levels.

20

Thus the present inventors have surprisingly found that the dimeric molecules of the present invention present a family of new compounds that are indicated for the treatment and prevention of a range of important human diseases and disorders set out above. These diseases and disorders include cancers, inflammatory disorders, autoimmune disorders,
25 cardiovascular disorders, and disorders associated with estrogen receptor activation.

In particular, the dimeric compounds of the present invention through their potent ability to inhibit proliferation of cancer cells and to induce apoptosis show use in the prevention and treatment of cancers, a term which any person skilled in the art would understand embraces
30 aberrant growth of both a benign and malignant nature of any or all tissues in the body across epithelial, mesenchymal and neural types. This includes, but is not restricted to,

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carcinomas, adenocarcinomas, sarcomas, blastomas, adenomas, lymphomas, leukaemias, gliomas and melanoma.

In particular, the dimeric compounds of the present invention through their potent ability to
5 act as h-ER β agonists are able to provide oestrogenic support in particular to women of
peri-menopausal and menopausal age and to prevent and to treat problems generally
recognised as representing acute withdrawal of steroidal estrogen such as vasomotor
symptoms (hot flushes and night sweats) and emotional symptoms (anxiety, depression,
mood swings), problems generally recognised as representing sub-acute withdrawal of
10 steroidal estrogen such as urinary incontinence and bladder prolapse, and problems
generally recognised as representing chronic withdrawal of steroidal estrogen such as
osteopaenia and osteoporosis, and senile dementia.

In particular, the dimeric compounds of the present invention through their potent ability to
15 act as h-ER α antagonists are able to prevent or to treat conditions in pre-menopausal
women generally regarded as being associated with excessive stimulation of h-
ER α receptors and leading to such conditions as cyclic mastalgia, endometriosis,
endometrial hyperplasia, uterine fibroids, polycystic ovarian disease, and pre-menstrual
syndrome.

20

In particular, the dimeric compounds of the present invention through their potent ability to
induce vasodilation and to reduce vasospasm show use in the treatment and prevention of
disorders generally recognised as being associated with vasopression either causally or
indirectly and embracing but not limited to hypertension and migraine headache.

25

In particular, the dimeric compounds of the present invention through their potent ability to
antagonise inflammatory processes and to moderate immunological processes show use in
the prevention and treatment of disorders generally recognised as being associated with
excessive inflammation or dysfunctional immune function and embracing but not limited
30 to inflammatory conditions of the gastrointestinal tract including inflammatory bowel

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disease, ulcerative colitis, Crohn's disease, and sclerosing cholangitis, and inflammatory disorders of synovial membranes including rheumatoid arthritis.

5 In particular, the dimeric compounds of the present invention through their potent ability to act as anti-oxidants show use in the prevention and treatment of conditions generally recognised as being associated with oxidation leading to degenerative changes and including but not limited to conditions such as cataracts, actinic damage, and atherosclerosis.

10 In particular, the dimeric compounds of the present invention through their potent ability to inhibit androgenesis show use in the prevention and treatment of conditions generally recognised as being associated (either causally or indirectly) with aberrant function of androgens and including but not limited to male pattern baldness (alopecia hereditaria) and prostatic adenoma.

15

The amount of one or more compounds of formulae I and II which is required in a therapeutic treatment according to the invention will depend upon a number of factors, which include the specific application, the nature of the particular compound used, the condition being treated, the mode of administration and the condition of the patient.

20 Compounds of formulae I or II may be administered in a manner and amount as is conventionally practised. See, for example, Goodman and Gilman, *The Pharmacological Basis of Therapeutics*, 1299 (7th Edition, 1985). The specific dosage utilised will depend upon the condition being treated, the state of the subject, the route of administration and other well known factors as indicated above. In general, a daily dose per patient may be in
25 the range of 0.1 mg to 2 g; typically from 0.5 mg to 1 g; preferably from 20 mg to 200 mg.

The production of pharmaceutical compositions for the treatment of the therapeutic indications herein described are typically prepared by admixture of the compounds of the invention (for convenience hereafter referred to as the "active compounds") with one or
30 more pharmaceutically or veterinarily acceptable carriers and/or excipients as are well known in the art.

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The carrier must, of course, be acceptable in the sense of being compatible with any other ingredients in the formulation and must not be deleterious to the subject. The carrier or excipient may be a solid or a liquid, or both, and is preferably formulated with the compound as a unit-dose, for example, a tablet, which may contain from 0.5% to 59% by weight of the active compound, or up to 100% by weight of the active compound. One or more active compounds may be incorporated in the formulations of the invention, which may be prepared by any of the well known techniques of pharmacy consisting essentially of admixing the components, optionally including one or more accessory ingredients.

10

The formulations of the invention include those suitable for oral, rectal, optical, buccal (for example, sublingual), parenteral (for example, subcutaneous, intramuscular, intradermal, or intravenous) and transdermal administration, although the most suitable route in any given case will depend on the nature and severity of the condition being treated and on the nature of the particular active compound which is being used.

15

Formulation suitable for oral administration may be presented in discrete units, such as capsules, sachets, lozenges, or tablets, each containing a predetermined amount of the active compound; as a powder or granules; as a solution or a suspension in an aqueous or non-aqueous liquid; or as an oil-in-water or water-in-oil emulsion. Such formulations may be prepared by any suitable method of pharmacy which includes the step of bringing into association the active compound and a suitable carrier (which may contain one or more accessory ingredients as noted above). In general, the formulations of the invention are prepared by uniformly and intimately admixing the active compound with a liquid or finely divided solid carrier, or both, and then, if necessary, shaping the resulting mixture such as to form a unit dosage. For example, a tablet may be prepared by compressing or moulding a powder or granules containing the active compound, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing, in a suitable machine, the compound of the free-flowing, such as a powder or granules optionally mixed with a binder, lubricant, inert diluent, and/or surface active/dispersing agent(s). Moulded

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tablets may be made by moulding, in a suitable machine, the powdered compound moistened with an inert liquid binder.

Formulations suitable for buccal (sublingual) administration include lozenges comprising
5 the active compound in a flavoured base, usually sucrose and acacia or tragacanth; and
pastilles comprising the compound in an inert base such as gelatin and glycerin or sucrose
and acacia.

Compositions of the present invention suitable for parenteral administration conveniently
10 comprise sterile aqueous preparations of the active compounds, which preparations are
preferably isotonic with the blood of the intended recipient. These preparations are
preferably administered intravenously, although administration may also be effected by
means of subcutaneous, intramuscular, or intradermal injection. Such preparations may
conveniently be prepared by admixing the compound with water or a glycine buffer and
15 rendering the resulting solution sterile and isotonic with the blood. Injectable formulations
according to the invention generally contain from 0.1% to 60% w/v of active compound
and are administered at a rate of 0.1 ml/minute/kg.

Formulations suitable for rectal administration are preferably presented as unit dose
20 suppositories. These may be prepared by admixing the active compound with one or more
conventional solid carriers, for example, cocoa butter, and then shaping the resulting
mixture.

Formulations or compositions suitable for topical administration to the skin preferably take
25 the form of an ointment, cream, lotion, paste, gel, spray, aerosol, or oil. Carriers that may
be used include Vaseline, lanoline, polyethylene glycols, alcohols, and combination of two
or more thereof. The active compound is generally present at a concentration of from
0.1% to 0.5% w/w, for example, from 0.5% to 2% w/w. Examples of such compositions
include cosmetic skin creams.

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Formulations suitable for transdermal administration may be presented as discrete patches adapted to remain in intimate contact with the epidermis of the recipient for a prolonged period of time. Such patches suitably contain the active compound as an optionally buffered aqueous solution of, for example, 0.1 M to 0.2 M concentration with respect to
5 the said active compound.

Formulations suitable for transdermal administration may also be delivered by iontophoresis (see, for example, *Panchagnula et al*) and typically take the form of an optionally buffered aqueous solution of the active compound. Suitable formulations
10 comprise citrate or Bis/Tris buffer (pH 6) or ethanol/water and contain from 0.1 M to 0.2 M active ingredient.

The active compounds may be provided in the form of food stuffs, such as being added to, admixed into, coated, combined or otherwise added to a food stuff. The term food stuff is
15 used in its widest possible sense and includes liquid formulations such as drinks including dairy products and other foods, such as health bars, desserts, etc. Food formulations containing compounds of the invention can be readily prepared according to standard practices.

20 Formulations suitable for inhalation may be delivered as a spray composition in the form of a solution, suspension or emulsion. The inhalation spray composition may further comprise a pharmaceutically acceptable propellant such as carbon dioxide or nitrous oxide.

The compositions of the invention may also be administered to a human in a dietary
25 supplement form. Dietary supplements incorporating the actives can be prepared by adding the composition to a food in the process of preparing the food. Any food may be used including, but not limited thereto, meats such as ground meats, emulsified meats and marinated meats; beverages such as nutritional beverages, sports beverages, protein fortified beverages, juices, milk, milk alternatives, and weight loss beverages; cheeses such
30 as hard and soft cheeses, cream cheese, and cottage cheese; frozen desserts such as ice cream, ice milk, low fat frozen desserts, and non-dairy frozen desserts; yogurts; soups;

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puddings; bakery products; salad dressings; and dips and spreads such as mayonnaise, margarine, butter, butter substitute, and other fat containing spreads. The composition is added to the food in an amount selected to deliver a desired dose of the composition to the consumer of the food.

5

Compounds of the present invention have potent antioxidant activity and thus find wide application in pharmaceutical and veterinary uses, in cosmetics such as skin creams to prevent skin ageing, in sun screens, in foods, health drinks, shampoos, and the like.

- 10 It has surprisingly been found that compounds of the formulae I or II interact synergistically with vitamin E to protect lipids, proteins and other biological molecules from oxidation.

- Accordingly a further aspect of this invention provides a composition comprising one or
15 more compounds of formulae I and II, vitamin E, and optionally a pharmaceutically, veterinarily or cosmetically acceptable carriers and/or excipients.

- Therapeutic methods, uses and compositions may be for administration to humans or animals, such as companion and domestic animals (such as dogs and cats), birds (such as
20 chickens, turkeys, ducks), livestock animals (such as cattle, sheep, pigs and goats) and the like.

- As used herein, the term "treatment" is to be considered in its broadest context. The term does not necessarily imply that an animal is treated until total recovery. Accordingly,
25 "treatment" includes amelioration of the symptoms or severity of a particular condition or preventing or otherwise reducing the risk of developing a particular condition.

- A "pharmaceutically acceptable carrier, excipient, auxiliary and/or diluent" as used herein should be taken to include any carrier, excipient, auxiliary or diluent that is considered
30 useful in preparing a pharmaceutical composition. Such carriers, excipients, auxiliaries or diluents will be generally safe, non-toxic and neither biologically nor otherwise

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undesirable. The term also includes carriers, excipients, auxiliaries or diluents that are acceptable for veterinary use as well as human pharmaceutical use. As used herein the term "pharmaceutically acceptable carriers, excipients, auxiliaries and/or diluents" includes one of, or more than one of, such substances.

5

The invention is further described with reference to the following non-limiting examples.

Examples

Example 1: Synthesis of tetra-acetoxy 6(3-(isoflavan-4',7-diol))-isoflav-3-ene-4',7-diol

10 (2)

A mixture of approximately 1:1 *cis*-and *trans*-diacetoxytetrahydrodaidzein (1) (300g) is dissolved in dry dichloromethane and stirred under a bed of nitrogen at 15°C until all material has dissolved. Phosphorous pentoxide (500g) is added quickly with vigorous stirring and the reaction is continued for 4-6 hours. The reaction mixture is then filtered
15 through a bed of silica gel with the dimeric structure having a lower R_f than the monomeric side product. The dimeric compound is further purified by selective recrystallisation from ethyl acetate.

^1H NMR (CDCl_3 , 400MHz): 7.40 (2H, d, $J=8\text{Hz}$), 7.15 (2H, d, $J=8\text{Hz}$), 7.13 (2H, d, $J=8\text{Hz}$), 7.05 (2H, d, $J=8\text{Hz}$), 6.90 (1H, d $J=7.6\text{Hz}$), 6.75 (1H, d, $J=1.5\text{Hz}$), 6.65 (1H, bs s), 6.63 (1H, br s), 6.60 (1H, dd, $J=7.6, 1.5\text{Hz}$), 6.58 (1H, br s), 5.05 (2H, brs), 4.38 (1H, dd, $J=9, 3\text{Hz}$), 4.30 (1H br d, $J=7\text{Hz}$), 4.25 (1H, dd, $J=9, 6\text{Hz}$), 3.38 (1H, m), 2.32 (3H, s), 2.30 (3H, s) 2.28 (3H, s), 2.08 (3H, br s)

25

Example 2: Synthesis of 6(3-(isoflavan-4',7-diol))-isoflav-3-ene-4',7-diol (3)

Imidazole (0.166g) was added to a suspension of tetra-acetoxy 6(3-(isoflavan-4',7-diol))-isoflav-3-ene-4',7-diol (0.2g) in absolute ethanol (2.0ml). The mixture was refluxed under nitrogen for 12 hours. The solution was concentrated under vacuum and the product was
30 precipitated by addition of deionised water (10ml). The mixture was left in the fridge

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overnight. The off-white solid was filtered and freeze-dried to afford (0.1g, 67%) of the free phenolic dimeric compound.

¹H NMR (D6-DMSO, 400MHz): 9.1 (4H, br, OH), 7.26 (2H, d, J=7.6Hz), 7.04 (2H, d, J=7.6Hz), 6.72 (2H, d, J=7.6Hz), 6.61 (1H, s), 6.60 (2H, d, J=7.6Hz), 6.56 (1H, s), 6.46 (1H, d, J=8Hz), 6.25 (1H, s), 6.22 (1H, brd, J=8Hz), 6.20 (1H, brs), 4.95 (2H, s), 4.44 (1H, d, J=8.4Hz), 4.13 (1H, dd, J=11, 3.3Hz), 4.06 (1H, dd, J=11, 7.7Hz), 3.28 (1H, ddd, J=8.4, 7.7, 3.3Hz).

10 Example 3: Estrogen Receptor Binding Assay

The ability of the tetraphenolic dimer (Structure 3) and tetraacetoxo dimer (Structure 2) to interact with estrogen receptor alpha and beta was determined using a commercially available estrogen receptor binding kit. This kit employs a competitive binding assay to determine the relative binding affinity of test compounds for recombinant human estrogen receptors-alpha (ER- α) and beta (ER- β). Briefly, recombinant human estrogen receptor alpha or beta is added to fluorescently labelled estrogen ligand to form an estrogen ligand/receptor complex which exhibits high fluorescence polarization. The complex is then added to decreasing concentrations of competitor test compounds. The shift in polarization in the presence of test compound is used to determine the relative affinity of test compounds for the estrogen receptor. When assayed using the manufacturer's specifications, we determined the concentration of estradiol required to displace 50% of bound fluorescent estrogen ligand (EC₅₀) at 0.011 μ M for ER-alpha and 0.006 μ M for ER-beta which is in agreement with published values (Bolger *et al.*, 1998). The tetraphenolic dimer (Structure 3) demonstrated equal binding affinity for estrogen receptor alpha and beta (ER- α 0.35 \pm 0.05 μ M; ER- β 0.37 \pm 0.05 μ M.), however the tetraacetoxo dimer (Structure 2) did not bind either receptor.

Example 4: Anticancer Activity

The anticancer potential of the tetraphenolic dimer (Structure 3) and the tetraacetoxo dimer (Structure 2) was assessed using a panel of human tumour cell lines, including glioma (C6), prostate (PC3), breast (MCF-7), soft tissue (Kym-1) and lung (NCI-H23 and NCI-

- 27 -

H460). The cell cytotoxicity assay was conducted as per the method of Alley *et al.*, 1988. Cell lines are exposed to varying concentrations of test compound for a fixed time. At the conclusion of the assay, the viability of the cell population is determined using the hydrogen acceptor reagent 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT). The concentration of compound required to inhibit 50% of cell growth in comparison to a control (IC₅₀) is an indicator of effectiveness of the compound. (*i.e.* the lower the concentration of compound required to inhibit 50% of normal cell proliferation, the more efficacious). Both dimers exhibited potent anticancer activity against the cancer types tested (Table 1).

10

Table 1. IC₅₀ determinations for both dimeric isoflavones against tumour cell lines.

| Dimer | Cell Line IC ₅₀ (μM) | | | | | |
|-------------|---------------------------------|------|--------------|-------|--------------|---------|
| | MCF-7 | PC3 | C6 Glioma | Kym-1 | NCI- H460 | NCI-H23 |
| Structure 3 | 18 ± 2 | 13.5 | 17.5 | 3.2 | 14.4 | 13.5 |
| Structure 2 | 10 ± 5 | 6 | NT | NT | NT | 7.3 |

NT = not tested

15

The reference to any prior art in this specification is not, and should not be taken as, an acknowledgment or any form of suggestion that that prior art forms part of the common general knowledge in the field of endeavour.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification individually or collectively, and any and all combinations of any two or more of said steps or features.

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References**Patents:**

JP 9067362-A A; Patent Assignee Sankyo Co Ltd; "New bis-iso-flavone(s) as 5-alpha-reductase inhibitors - prepared from Microbiospora, used for treatment of
5 prostatomegally."

WO 00/49009; Patent assignee Novogen Research Pty Ltd; "Production of Isoflavone derivatives"

10 WO 91/14429; Chiesi P and Pavesi L Patent; Assignee: Chiesi; "Pharmaceutical compositions containing ipriflavone, process for the preparation thereof and relative therapeutic use."

WO 93/23069; Kelly "GE Health Supplements".

15

WO 96/10341; Schouten Food and Health Products

US 5424331; Shlyankevich; "Pharmaceutical Compositions and Dietary Soybean Food Products for the Prevention of Osteoporosis".

20

JP 62-106017; Yamanouchi Pharmaceuticals Co Ltd; "Anticancer agent for all isoflavonoid agents".

JP 62-106016; Yamanouchi Pharmaceuticals Co Ltd; "Therapeutic claims over
25 isoflavonoids as immuno-suppressive".

US 5516528; Hughes et al; "Dietary phytoestrogen in estrogen replacement therapy".
(Assignees: Wake Forest Uni and PTI)

- 29 -

JP 62-106016A2; Immunosuppressor; Yamanouchi Pharmaceutical Co Ltd; "Isoflavones for remedy and the prevention of relapse of autoimmune diseases such as rheumatoid arthritis, SLE etc".

- 5 WO 01/17986; Novogen Research; Compositions and therapeutic methods involving isoflavones."

JP 62-106017A2; Anti-tumor agent; Yamanouchi Pharmaceut. Co Ltd; "oncogene originated tyrosine-specific phosphorylase-inhibiting activity".

10

JP 61-246124; A Carcinostatic Agent; Yamanouchi Pharmaceut. Co Ltd; "carcinostatic agent containing genistein".

- 15 WO 98/50026; Kelly GE; "Treatment or prevention of menopausal symptoms or osteoporosis – comprises administering the iso-flavone(s) formononetin or daidzein".

WO 99/43335; Huang LJ and Thurn MJ; "Plant extracts from clover"

WO 00/49009; Heaton A and Kumar N; "Production of isoflavone derivatives".

20

WO 00/644438; Husband AJ and Kelly GE; "Composition for treatment of cardiovascular diseases and osteoporosis comprises high proportion of formononetin with biochanin, genistein and/or genistein".

- 25 WO 99/48496; Husband AJ and Kelly GE; "Treating disorders associated with high steroidal estrogen activity e.g. uterine fibroids".

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- 30 -

Articles:

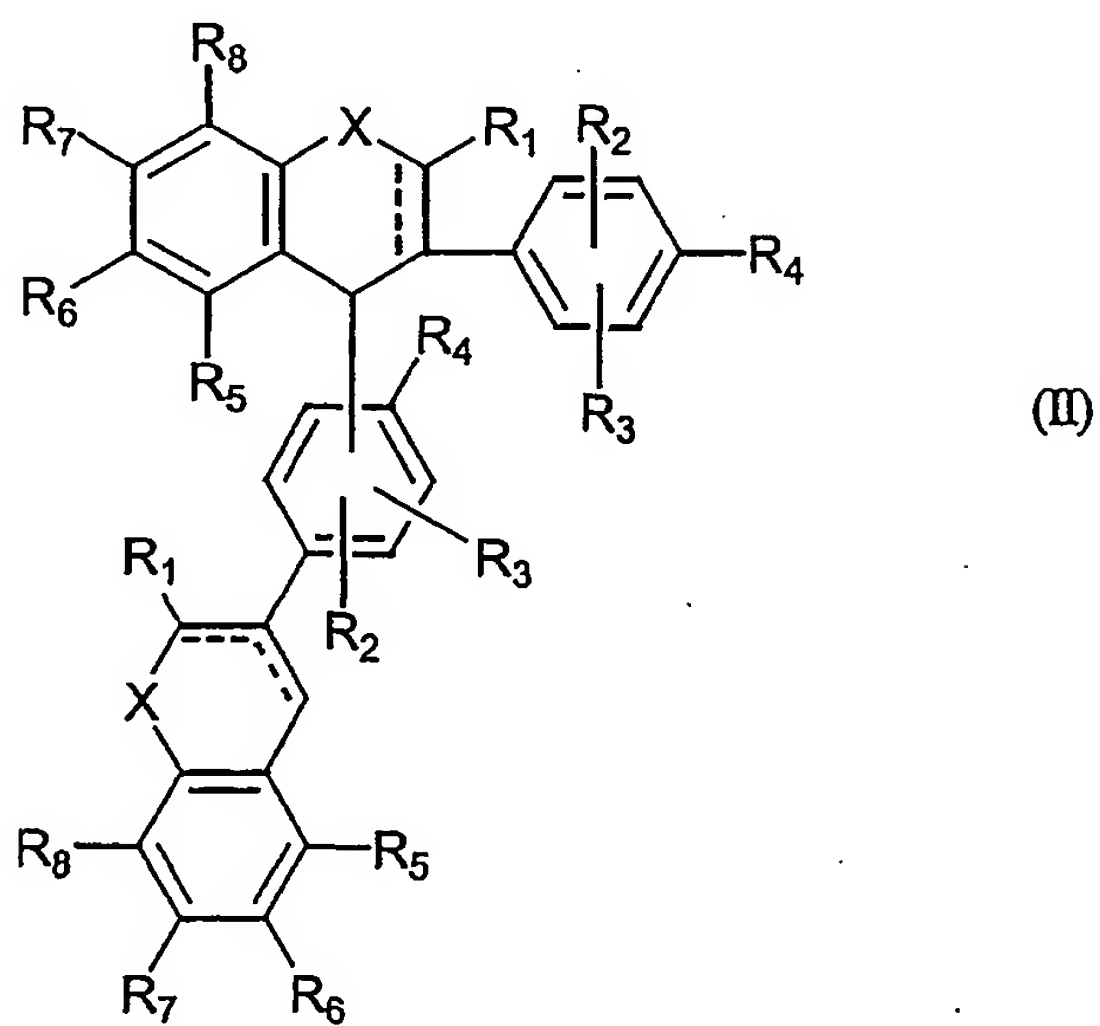
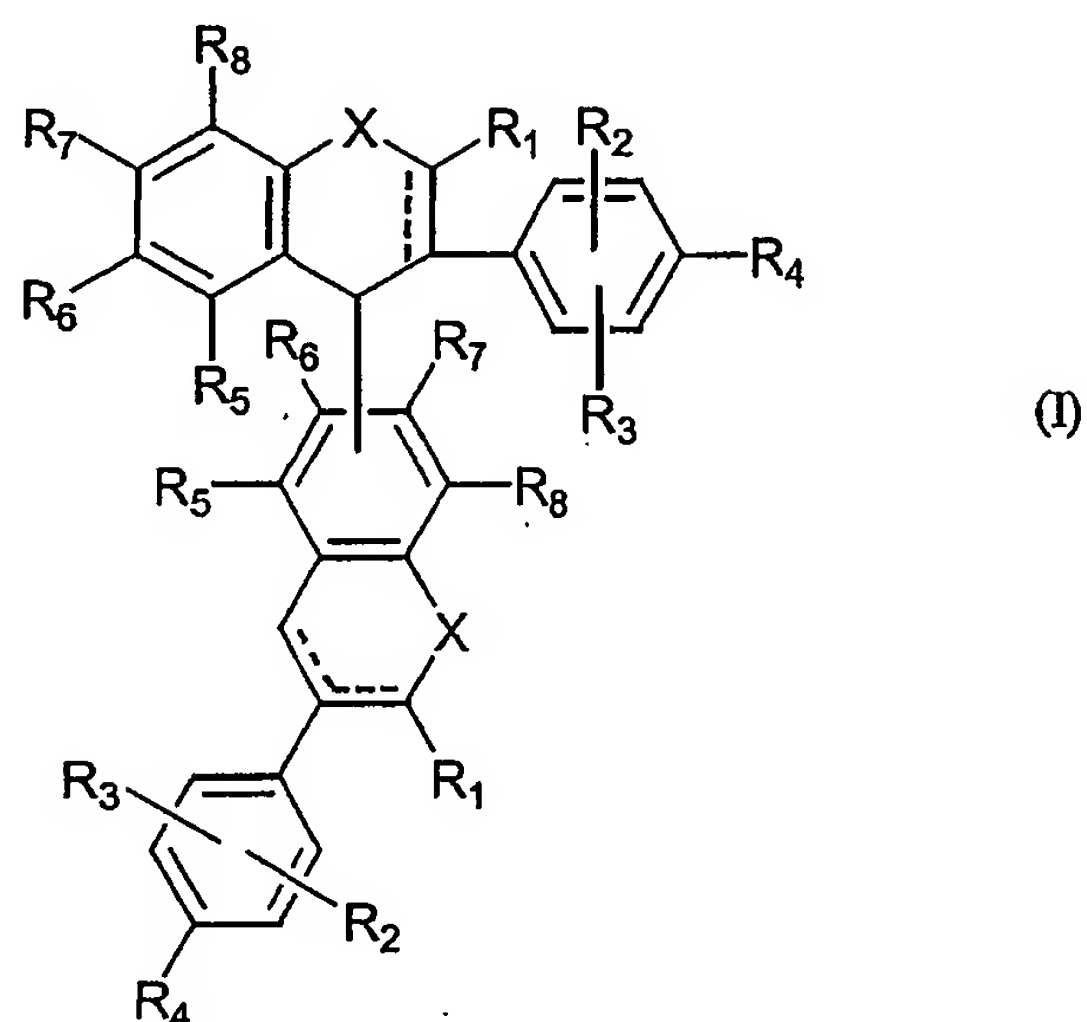
- Alley MC, Scudiero DA, Monks A, Hursey ML, Czerwinski MJ, Fine DL, Abbott BJ, Mayo JG, Shoemaker RH, and Boyd MR. 1988. Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay. *Cancer Res.* 48, 589-601.
- Al-Maharik NI, Kaltia SAA, Mutikainen I, Wahala K Synthesis of C-C-Bridged Bis-Isoflavones *Journal of Organic Chemistry* 65, 2305-2308, 2000
- 10 Bingham SA, Atkinson C, Liggins J, Bluck L, Coward A. Phyto-oestrogens: where are we now? *British Journal Of Nutrition* 79(5): 393-406 1998
- Bolger R, Wiese TE, Ervin K, Nestich S, Checovich W (1998) Rapid screening of environmental chemicals for estrogen receptor binding capacity. *Environ Health Perspect;* 106, 551-7.
- Goodman and Gilman, *The Pharmacological Basis of Therapeutics*, 1299 7th Edition, 1985
- 20 Moss RW, Cancer Therapy: the Independent Consumer's Guide to Non-Toxic Treatment and Prevention (New York: Equinox Press, 1992)
- Panchagnula *et al.* (2000) Transdermal iontophoresis revisited. *Current Opinion in Chemical Biology* 4: 468-473
- 25 Scudiero DA, Shoemaker RH, Paull KD, Monks A, Tierney S, Nofziger TH, Currans MJ, Seniff D, Boyd MR. (1988) Evaluation of a soluble tetrazolium/formazan assay for cell growth and drug sensitivity in culture using human and other

- 31 -

Shen F, Xue X, Weber G. Tamoxifen and genistein synergistically down-regulate signal transduction and proliferation in estrogen receptor-negative human breast carcinoma MDA-MB-435 cells *Anticancer Research* 1657-1662 1999

CLAIMS:

1. A compound of the general formulae (I) or (II):



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wherein

$R_1, R_2, R_3, R_4, R_5, R_6, R_7$ and R_8 are independently hydrogen, hydroxy, OR_9 , $OC(O)H$, $OC(O)R_9$, $OS(O)R_9$, $OSi(R_{10})_3$, $C(O)R_{11}$, CO_2R_{12} , alkyl, haloalkyl, aryl, arylalkyl, thio, alkylthio, amino, alkylamino, dialkylamino, nitro or halo,

R_9 is alkyl, haloalkyl, aryl, arylalkyl or alkylaryl,

R_{10} is independently hydrogen, alkyl or aryl,

R_{11} is hydrogen, alkyl, aryl, arylalkyl or an amino acid, and

R_{12} is hydrogen, alkyl, haloalkyl, aryl or arylalkyl,

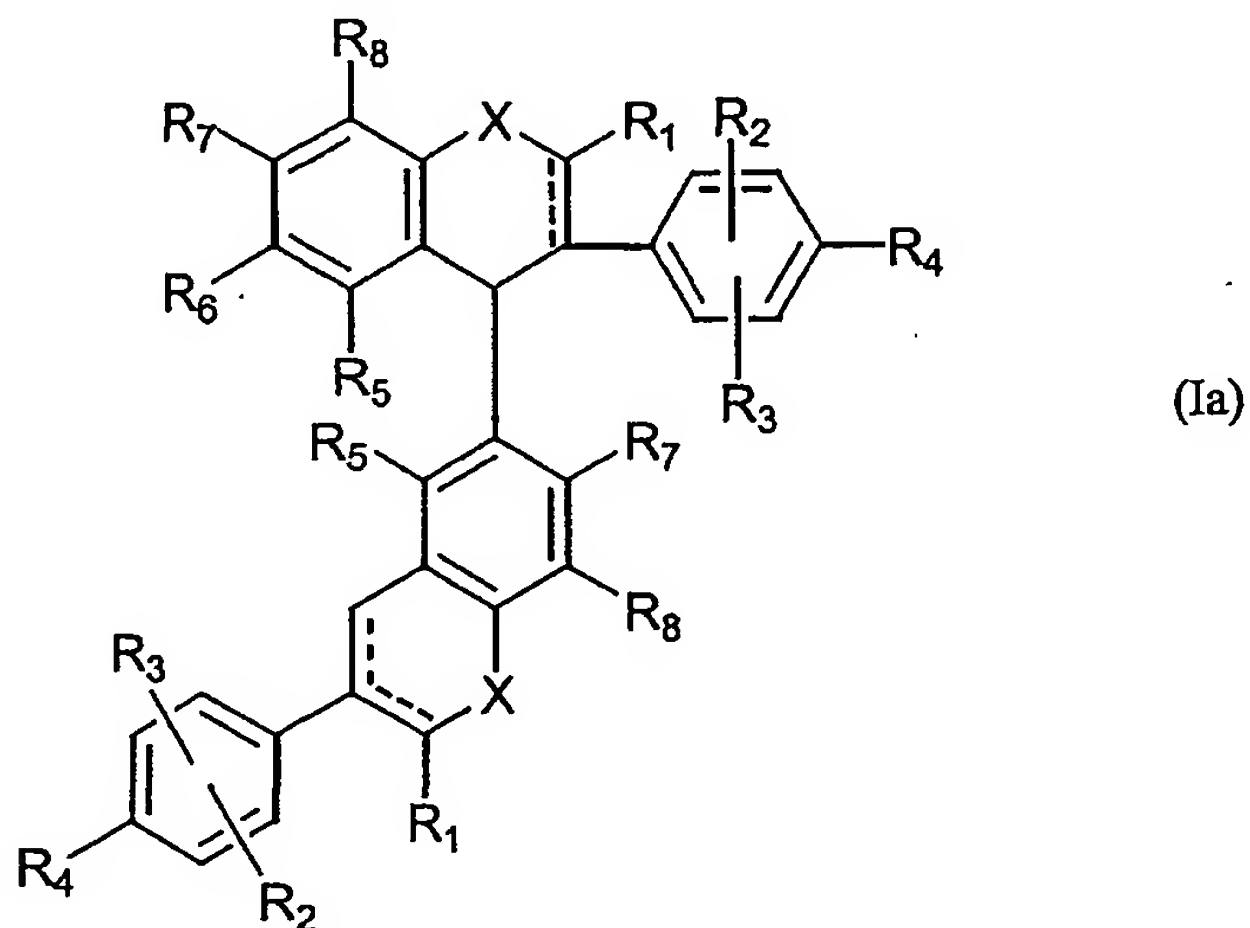
X is O, NR_4 or S, and

the drawing "—" represents either a single bond or a double bond,

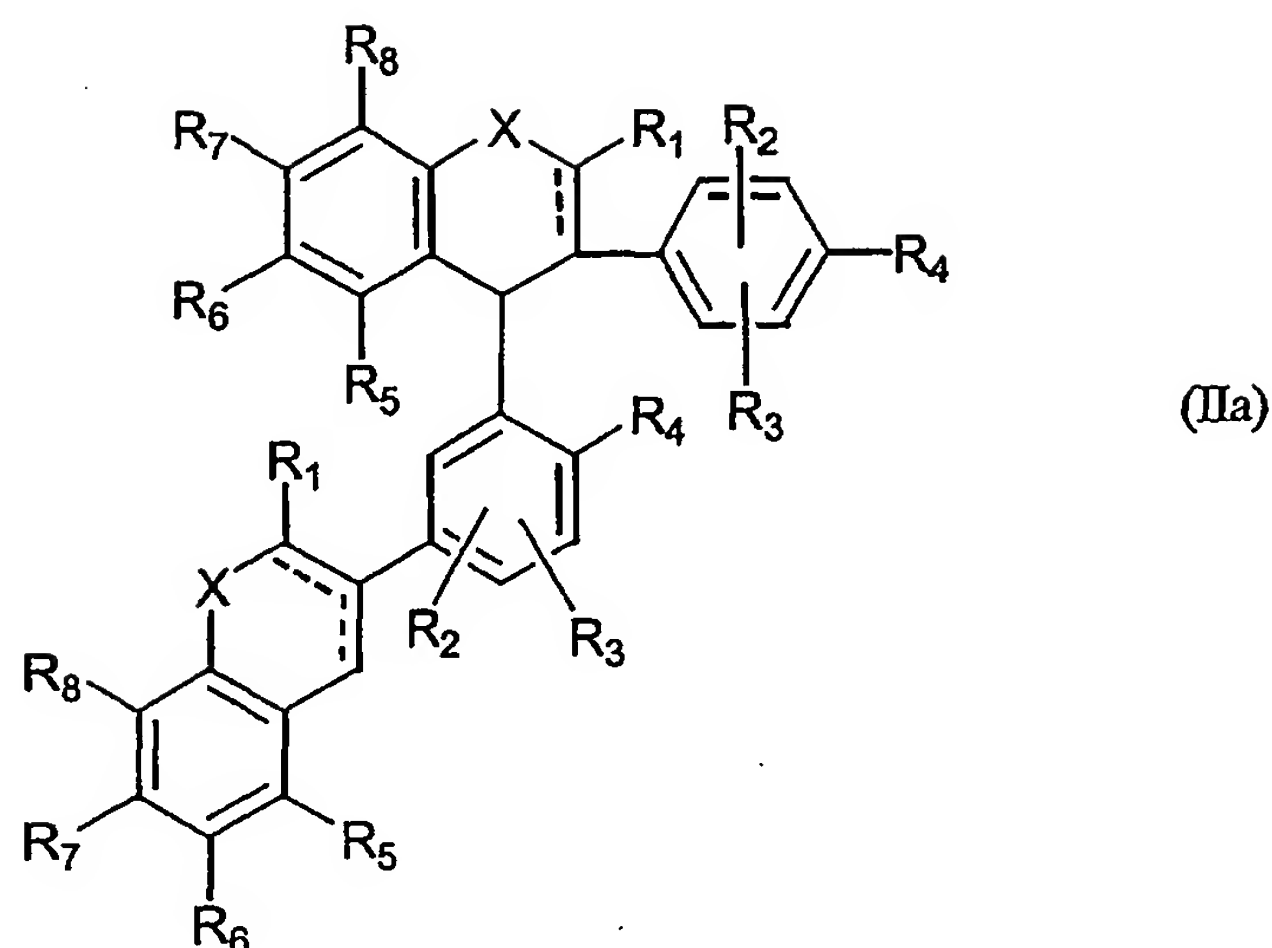
which compounds include pharmaceutically acceptable salts thereof,

with the proviso that in formula (I) at least one of R_5, R_6, R_7 and R_8 is hydrogen.

2. A compound of claim 1 selected from the compounds of general formulae (Ia) and (IIa):



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wherein

R_1 , R_2 , R_3 , R_4 , R_5 , R_6 , R_7 and R_8 are independently hydrogen, hydroxy, OR_9 , $OC(O)H$, $OC(O)R_9$, $OS(O)R_9$, $OSi(R_{10})_3$, $C(O)R_{11}$, CO_2R_{12} , alkyl, haloalkyl, aryl, arylalkyl, thio, alkylthio, amino, alkylamino, dialkylamino, nitro or halo,

R_9 is alkyl, haloalkyl, aryl, arylalkyl or alkylaryl,

R_{10} is independently hydrogen, alkyl or aryl,

R_{11} is hydrogen alkyl, aryl, arylalkyl or an amino acid, and

R_{12} is hydrogen, alkyl, haloalkyl, aryl or arylalkyl,

X is O, NR_4 or S, and

the drawing "----" represents either a single bond or a double bond,

which compounds include pharmaceutically acceptable salts thereof,

with the proviso that in Formula (Ia) at least one of R_5 , R_6 , R_7 and R_8 is hydrogen.

3. A compound of claim 2, wherein

R_1 , R_2 , R_3 , R_4 , R_5 , R_7 and R_8 are independently hydrogen, hydroxy, OR_9 , $OC(O)R_9$, $OS(O)R_9$, alkyl, aryl, arylalkyl, thio, alkylthio, bromo, chloro or fluoro,

R_6 is hydrogen,

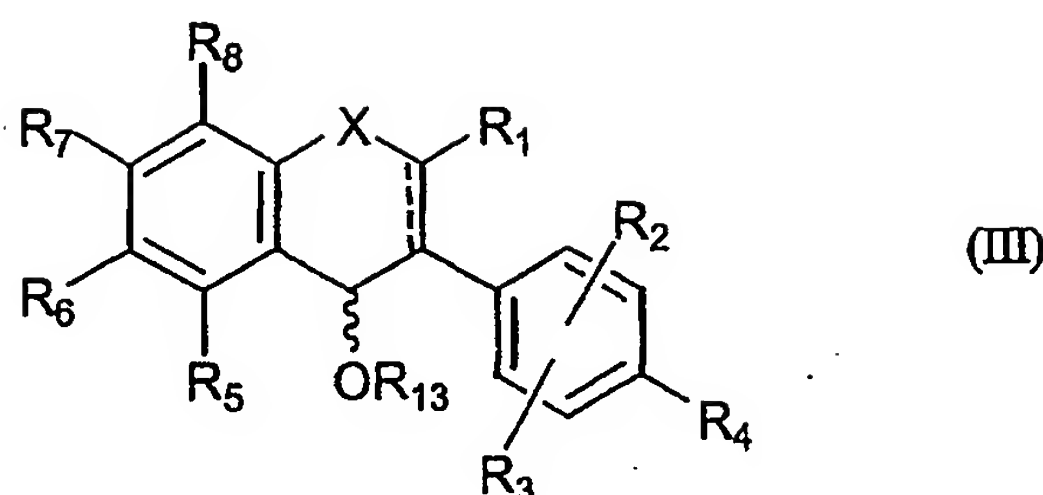
R_9 is alkyl, fluoroalkyl or arylalkyl, and

X is O.

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4. A compound of claim 3, wherein
R₁ and R₆ are hydrogen,
R₂, R₃, R₅ and R₈ are independently hydrogen, hydroxy, OR₉, OC(O)R₉, alkyl, aryl or arylalkyl,
R₄ and R₇ are independently hydroxy, OR₉ or OC(O)R₉,
R₉ is methyl, ethyl, propyl, isopropyl or trifluoromethyl, and
X is O.
5. A compound of claim 4, wherein
R₁ and R₆ are hydrogen,
R₂, R₃, R₅ and R₈ are independently hydrogen, hydroxy, OR₉, OC(O)R₉ or methyl,
R₄ and R₇ are independently hydroxy, OR₉ or OC(O)R₉,
R₉ is methyl, and
X is O.
6. A compound of claim 5 selected from:
Tetraacetoxy 6-(4-(isoflavan-4',7-diol))-isoflav-3-ene-4',7-diol (2)
6-(4-(Isoflavan-4',7-diol))-isoflav-3-ene-4',7-diol (3)
Tetraacetoxy 6-(4-(isoflavan-4',7-diol))-isoflavan-4',7-diol (4)
6-(4-(Isoflavan-4',7-diol))-isoflavan-4',7-diol (5)
6-(4-(4',7-Dimethoxyisoflavan))-4',7-dimethoxyisoflav-3-ene (6)
6-(4-(4',7-Dimethoxyisoflavan))-4',7-dimethoxyisoflavan (7)
Tetraacetoxy 3'-(4-(isoflavan-4',7-diol))-isoflav-3-ene-4',7-diol (8)
3'-(4-(Isoflavan-4',7-diol))-isoflav-3-ene-4',7-diol (9)
Tetraacetoxy 3'-(4-(isoflavan-4',7-diol))-isoflavan-4',7-diol (10)
3'-(4-(Isoflavan-4',7-diol))-isoflavan-4',7-diol (11).
7. A process for the preparation of a compound of formulae (I) or (II) which process comprises reacting a compound of the formula (III)

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wherein

R₁, R₂, R₃, R₄, R₅, R₆, R₇ and R₈ are independently hydrogen, hydroxy, OR₉, OC(O)H, OC(O)R₉, OS(O)R₉, OSi(R₁₀)₃, C(O)R₁₁, CO₂R₁₂, alkyl, haloalkyl, aryl, arylalkyl, thio, alkylthio, amino, alkylamino, dialkylamino, nitro or halo,

R₉ is alkyl, haloalkyl, aryl, arylalkyl or alkylaryl,

R₁₀ is independently hydrogen, alkyl or aryl,

R₁₁ is hydrogen, alkyl, aryl, arylalkyl or an amino acid, and

R₁₂ is hydrogen, alkyl, haloalkyl, aryl or arylalkyl,

OR₁₃ is hydroxy, another leaving group or an electron withdrawing group,

X is O, NR₄ or S, and

the drawing "—" represents either a single bond or a double bond,

which compounds include pharmaceutically acceptable salts thereof,

with the proviso that in the preparation of compounds of formula (I) at least one of R₅, R₆, R₇ and R₈ is hydrogen,

with a coupling agent.

8. A process of claim 7, wherein the compound of formulae (I) or (II) is as defined in any one of claims 2 to 6.

9. A process of claim 7 or 8, wherein the coupling agent is phosphorus pentoxide, hydrogen peroxide, permanganate or a Lewis acid.

10. A method for the treatment, prophylaxis or amelioration of a disease or disorder which method includes the step of administering a therapeutically effective amount of one

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or more compounds of formulae (I) and (II) to a subject, wherein the disease or disorder is selected from the group selected from

- a) all forms of cancer (pre-malignant, benign and malignant) in all tissues of the body. In this regard, the compounds may be used as the sole form of anti-cancer therapy or in combination with other forms of anti-cancer therapy including but not limited to radiotherapy and chemotherapy;
- b) diseases and disorders associated with inflammatory reactions of an abnormal or prolonged nature in any of the body's tissues including but not limited to rheumatoid arthritis, tendonitis, inflammatory bowel disease, ulcerative colitis, Crohn's Disease, sclerosing cholangitis;
- c) papulonodular skin lesions including but not limited to sarcoidosis, angiosarcoma, Kaposi's sarcoma, Fabry's Disease
- d) papulosquamous skin lesions including but not limited to psoriasis, Bowen's Disease, and Reiter's Disease;
- e) actinic damage characterized by degenerative changes in the skin including but not limited to solar keratosis, photosensitivity diseases, and wrinkling;
- f) diseases and disorders associated with abnormal angiogenesis affecting any tissue within the body including but not limited to hemangiomas and telangiectasia;
- g) proliferative disorders of bone marrow including but not limited to megaloblastic disease, myelodysplastic syndromes, polycythemia vera, thrombocytosis and myelofibrosis;
- h) autoimmune disease characterized by abnormal immunological responses including but not limited to multiple sclerosis, Type 1 diabetes, systemic lupus erythematosus, and biliary cirrhosis;
- i) neurodegenerative diseases and disorders characterized by degenerative changes in the structure of the neurological system including but not limited to Parkinson's Disease, Alzheimer's Disease, muscular dystrophy, Lou-Gehrig Disease, motorneurone disease;
- j) diseases and disorders associated with degenerative changes within the walls of blood vessels including but not limited to atherosclerosis, atheroma, coronary

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- artery disease, stroke, myocardial infarction, hypertensive vascular disease, malignant hypertension, thromboangiitis obliterans, fibromuscular dysplasia;
- k) diseases and disorders associated with abnormal immunological responses including but limited to dermatomyositis and scleroderma;
 - l) diseases and disorders associated with degenerative changes within the eye including but not limited to cataracts, macular degeneration, retinal atrophy.
11. A method of claim 10, wherein the disorder or disease is selected from:
- (i) conditions in women associated with abnormal estrogen/androgen balance including but not limited to cyclical mastalgia, acne, dysmenorrhoea, uterine fibroids, endometriosis, ovarian cysts, premenstrual syndrome, acute menopause symptoms, osteoporosis, senile dementia, infertility; and
 - (ii) conditions in men associated with abnormal estrogen/androgen balance including but not limited to benign prostatic hypertrophy, infertility, gynecomastia, alopecia hereditaria and various other forms of baldness.
12. Use of one or more compounds of formulae (I) and (II) in the manufacture of a medicament for the treatment of a disorder or disease.
13. Use of one or more compounds of formulae (I) and (II) as an anti-estrogen or selective estrogen receptor modulator (SERM).
14. An agent for the treatment, prophylaxis or amelioration of a disease or disorder which agent comprises one or more compounds of formulae (I) or (II).
15. A pharmaceutical composition which comprises one or more compounds of formulae (I) and (II) in association with one or more pharmaceutical carriers and/or excipients.
16. A drink or food-stuff, which contains one or more compounds of formulae (I) and (II).

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17. A composition comprising one or more compounds of formulae (I) and (II), vitamin E, and optionally a pharmaceutically, veterinarily or cosmetically acceptable carriers and/or excipients.

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undesirable. The term also includes carriers, excipients, auxiliaries or diluents that are acceptable for veterinary use as well as human pharmaceutical use. As used herein the term "pharmaceutically acceptable carriers, excipients, auxiliaries and/or diluents" includes one of, or more than one of, such substances.

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The invention is further described with reference to the following non-limiting examples.

Examples

Example 1: Synthesis of tetra-acetoxy 6(4-(isoflavan-4',7-diol))-isoflav-3-ene-4',7-diol

10 (2)

A mixture of approximately 1:1 *cis*-and *trans*-diacetoxytetrahydrodaidzein (1) (300g) is dissolved in dry dichloromethane and stirred under a bed of nitrogen at 15°C until all material has dissolved. Phosphorous pentoxide (500g) is added quickly with vigorous stirring and the reaction is continued for 4-6 hours. The reaction mixture is then filtered through a bed of silica gel with the dimeric structure having a lower R_f than the monomeric side product. The dimeric compound is further purified by selective recrystallisation from ethyl acetate.

¹H NMR (CDCl₃, 400MHz): 7.40 (2H, d, J=8Hz), 7.15 (2H, d, J=8Hz), 7.13 (2H, d, J=8Hz), 7.05 (2H, d, J=8Hz), 6.90 (1H, d J=7.6Hz), 6.75 (1H, d, J=1.5Hz), 6.65 (1H, bs s), 6.63 (1H, br s), 6.60 (1H, dd, J=7.6, 1.5Hz), 6.58 (1H, br s), 5.05 (2H, brs), 4.38 (1H, dd, J=9,3Hz), 4.30 (1H br d, J=7Hz), 4.25 (1H, dd, J=9,6Hz), 3.38 (1H, m), 2.32 (3H, s), 2.30 (3H, s) 2.28 (3H, s), 2.08 (3H, br s)

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Example 2: Synthesis of 6(4-(isoflavan-4',7-diol))-isoflav-3-ene-4',7-diol (3)

Imidazole (0.166g) was added to a suspension of tetra-acetoxy 6(4-(isoflavan-4',7-diol))-isoflav-3-ene-4',7-diol (0.2g) in absolute ethanol (2.0ml). The mixture was refluxed under nitrogen for 12 hours. The solution was concentrated under vacuum and the product was precipitated by addition of deionised water (10ml). The mixture was left in the fridge

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Articles:

Alley MC, Scudiero DA, Monks A, Hursey ML, Czerwinski MJ, Fine DL, Abbott BJ, Mayo JG, Shoemaker RH, and Boyd MR. 1988. Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay. *Cancer Res.* 48, 589-601

Al-Maharik NI, Kaltia SAA, Mutikainen I, Wahala K Synthesis of C-C-Bridged Bis-Isoflavones *Journal of Organic Chemistry* 65, 2305-2308, 2000

Bingham SA, Atkinson C, Liggins J, Bluck L, Coward A. Phyto-oestrogens: where are we now? *British Journal Of Nutrition* 79(5): 393-406 1998

Bolger R, Wiese TE, Ervin K, Nestich S, Checovich W (1998) Rapid screening of environmental chemicals for estrogen receptor binding capacity. *Environ Health Perspect*; 106, 551-7

Chang Y-C, Nair MG, Santell RC, and Helferich WG. Microwave-mediated synthesis of anticarcinogenic isoflavones from soybeans. *J Agric Food Chem.* 1994, 42, 1869-1871

Goodman and Gilman, *The Pharmacological Basis of Therapeutics*, 1299 7th Edition, 1985

Moss RW, *Cancer Therapy: the Independent Consumer's Guide to Non-Toxic Treatment and Prevention* (New York: Equinox Press, 1992)

Panchagnula *et al.* (2000) Transdermal iontophoresis revisited. *Current Opinion in Chemical Biology* 4: 468-473

Scudiero DA, Shoemaker RH, Paull KD, Monks A, Tierney S, Nofziger TH, Currens MJ, Seniff D, Boyd MR. (1988) Evaluation of a soluble tetrazolium/formazan assay for cell growth and drug sensitivity in culture using human and other cell line. *Cancer Res.* 48(17); 4827-33.

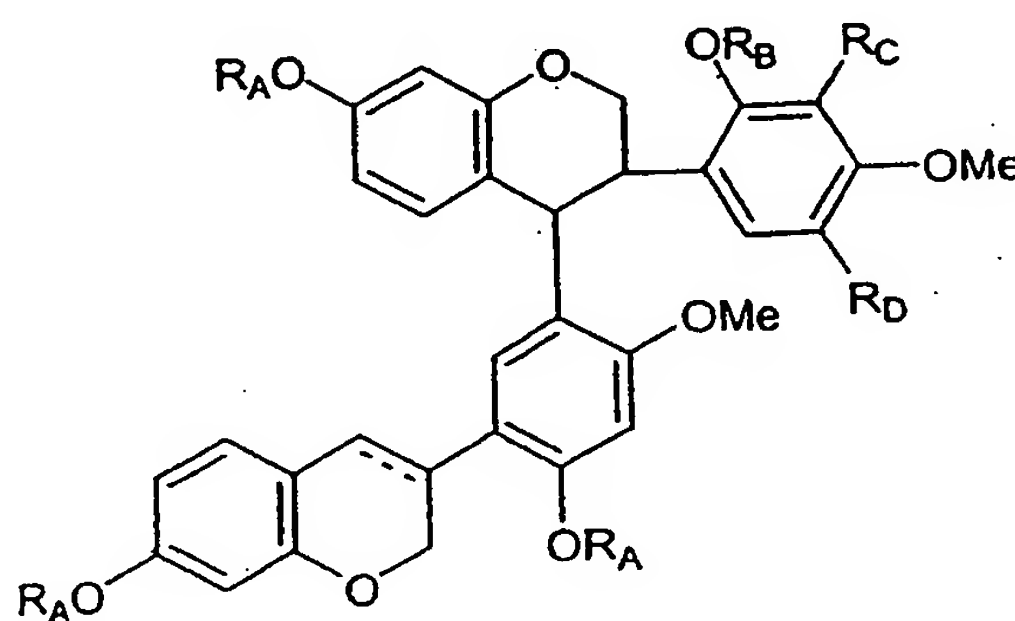
- 33 -

wherein

- $R_1, R_2, R_3, R_4, R_5, R_6, R_7$ and R_8 are independently hydrogen, hydroxy, OR_9 , $OC(O)H$, $OC(O)R_9$, $OS(O)R_9$, $OSi(R_{10})_3$, $C(O)R_{11}$, CO_2R_{12} , alkyl, haloalkyl, aryl, arylalkyl, thio, alkylthio, amino, alkylamino, dialkylamino, nitro or halo,
- R_9 is alkyl, haloalkyl, aryl, arylalkyl or alkylaryl,
- R_{10} is independently hydrogen, alkyl or aryl,
- R_{11} is hydrogen, alkyl, aryl, arylalkyl or an amino acid, and
- R_{12} is hydrogen, alkyl, haloalkyl, aryl or arylalkyl,
- X is O, NR_4 or S, and

the drawing "—" represents either a single bond or a double bond,
which compounds include pharmaceutically acceptable salts thereof,
with the proviso that in formula (I) at least one of R_5, R_6, R_7 and R_8 is hydrogen, and

with the proviso that compounds of the formula:



wherein

when "—" is a single bond or a double bond, and

R_A and R_B are hydrogen or methyl,

then R_C and R_D are hydrogen, and

when "—" is a single bond and R_A is hydrogen,

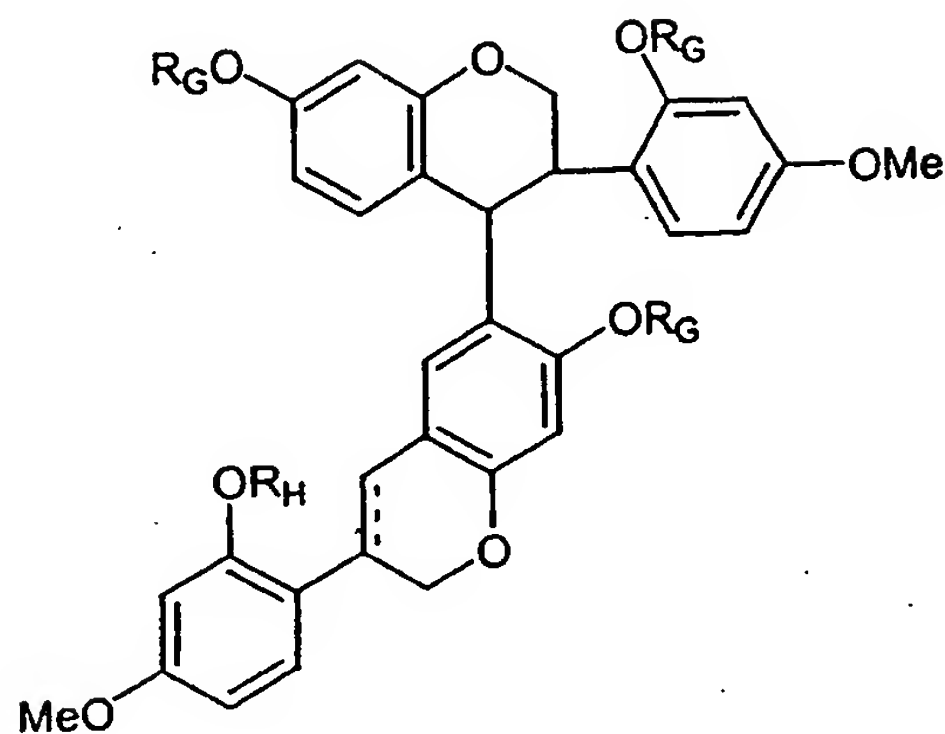
then either R_B is hydrogen or methyl, R_C is hydroxy and R_D is hydrogen, or

R_B and R_C are hydrogen and R_D is methoxy,

are specifically excluded, and

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with the proviso that compounds of the formula:



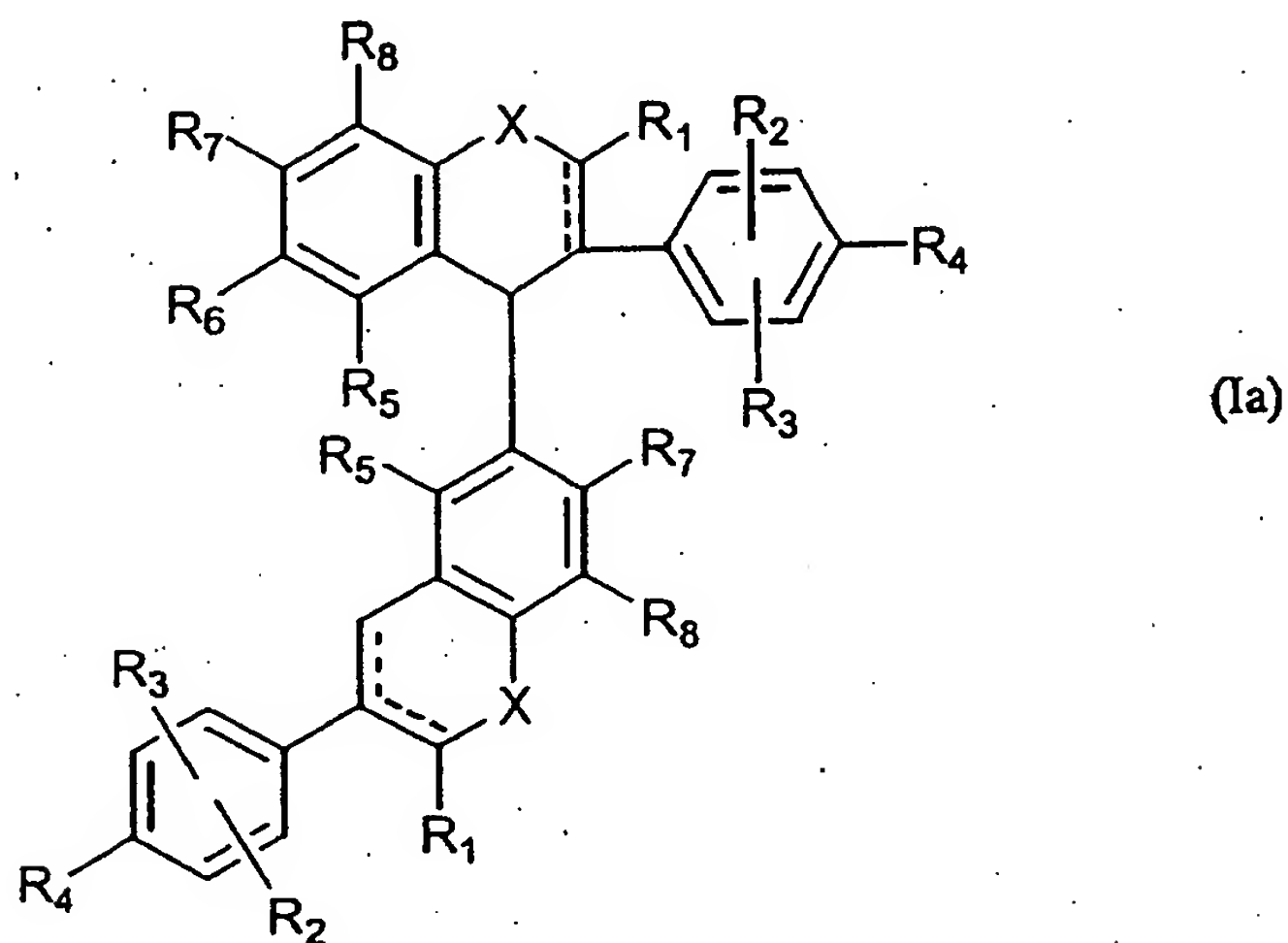
wherein

when " $---$ " is a single bond or a double bond,
then R_G and R_H are hydrogen or methyl, and

when " $---$ " is a single bond and R_G is methoxy,
then R_H is hydroxy or benzyl,
are specifically excluded.

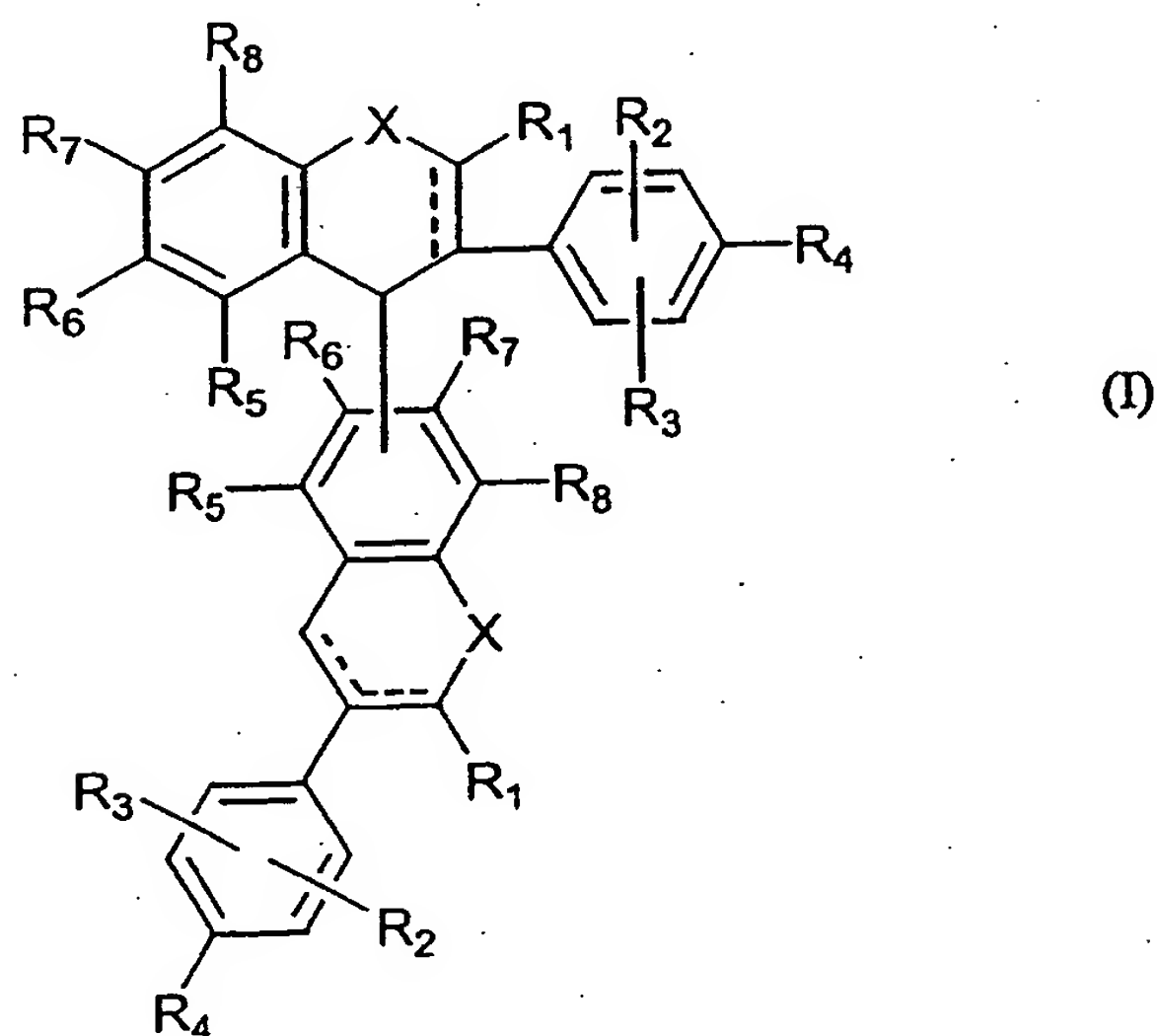
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2. A compound of claim 1 selected from the compounds of general formulae (Ia) and (IIa):

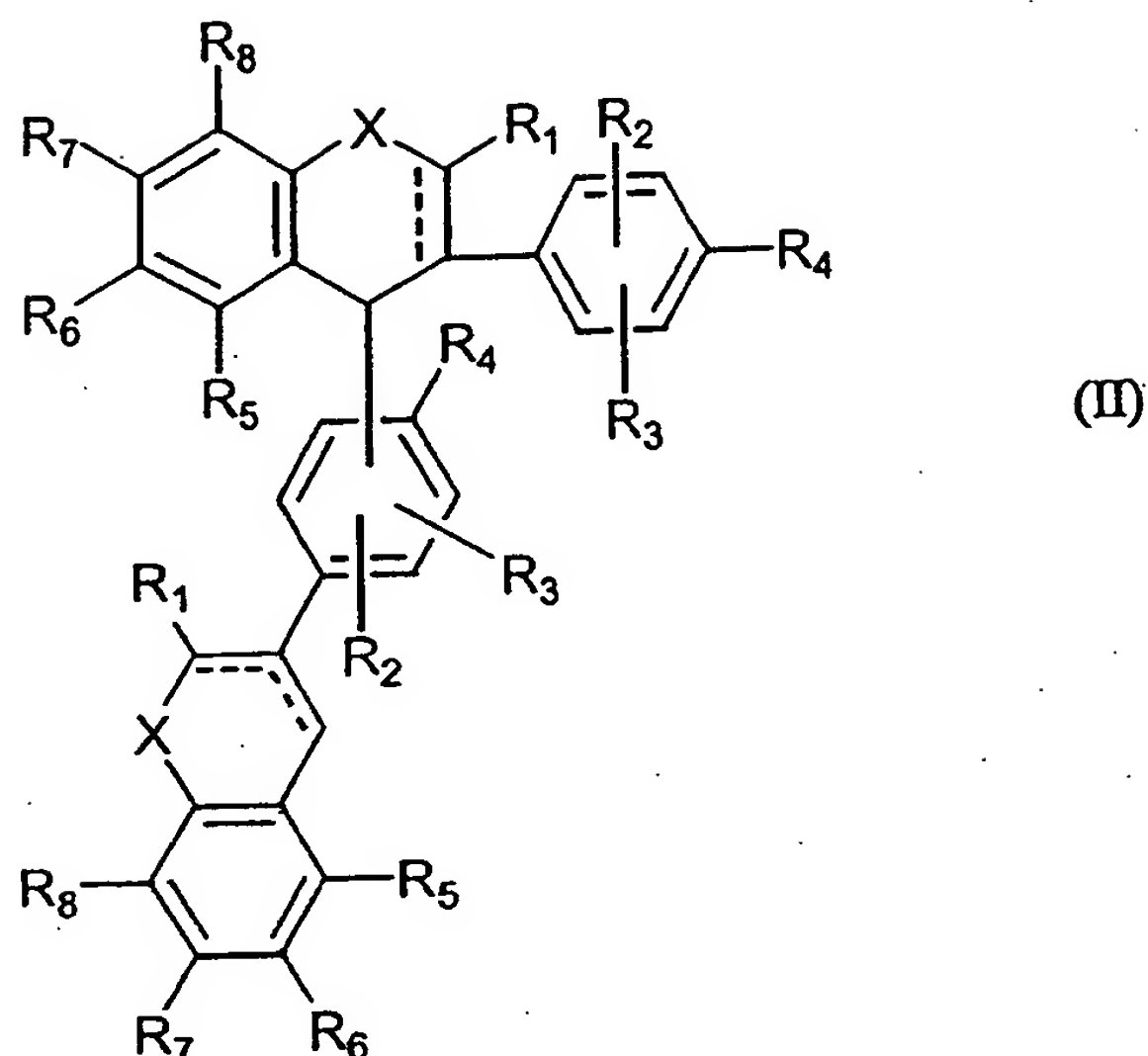


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- artery disease, stroke, myocardial infarction, hypertensive vascular disease, malignant hypertension, thromboangiitis obliterans, fibromuscular dysplasia;
- k) diseases and disorders associated with abnormal immunological responses including but limited to dermatomyositis and scleroderma;
- l) diseases and disorders associated with degenerative changes within the eye including but not limited to cataracts, macular degeneration, retinal atrophy,
- and wherein the one or more compounds of formulae (I) and (II) are:



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wherein

- $R_1, R_2, R_3, R_4, R_5, R_6, R_7$ and R_8 are independently hydrogen, hydroxy, OR_9 , $OC(O)H$, $OC(O)R_9$, $OS(O)R_9$, $OSi(R_{10})_3$, $C(O)R_{11}$, CO_2R_{12} , alkyl, haloalkyl, aryl, arylalkyl, thio, alkylthio, amino, alkylamino, dialkylamino, nitro or halo,
- R_9 is alkyl, haloalkyl, aryl, arylalkyl or alkylaryl,
- R_{10} is independently hydrogen, alkyl or aryl,
- R_{11} is hydrogen, alkyl, aryl, arylalkyl or an amino acid, and
- R_{12} is hydrogen, alkyl, haloalkyl, aryl or arylalkyl,
- X is O, NR_4 or S, and

the drawing "—" represents either a single bond or a double bond,
which compounds include pharmaceutically acceptable salts thereof,
with the proviso that in formula (I) at least one of R_5, R_6, R_7 and R_8 is hydrogen.

11. A method for the treatment, prophylaxis or amelioration of a disease or disorder which method includes the step of administering a therapeutically effective amount of one or more compounds of formulae (I) and (II) as defined in claim 10 to a subject, wherein the disease or disorder is selected from the group selected from:

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- (i) conditions in women associated with abnormal estrogen/androgen balance including but not limited to cyclical mastalgia, acne, dysmenorrhoea, uterine fibroids, endometriosis, ovarian cysts, premenstrual syndrome, acute menopause symptoms, osteoporosis, senile dementia, infertility; and
 - (ii) conditions in men associated with abnormal estrogen/androgen balance including but not limited to benign prostatic hypertrophy, infertility, gynecomastia, alopecia hereditaria and various other forms of baldness.
12. Use of one or more compounds of formulae (I) and (II) as defined in claim 10 in the manufacture of a medicament for the treatment of a disorder or disease.
13. Use of one or more compounds of formulae (I) and (II) as defined in claim 10 as an anti-estrogen or selective estrogen receptor modulator (SERM).
14. An agent for the treatment, prophylaxis or amelioration of a disease or disorder which agent comprises one or more compounds of formulae (I) or (II) as defined in claim 10.
15. A pharmaceutical composition which comprises one or more compounds of formulae (I) and (II) as defined in claim 10 in association with one or more pharmaceutical carriers and/or excipients.
16. A drink or food-stuff, which contains one or more compounds of formulae (I) and (II) as defined in claim 10.
17. A composition comprising one or more compounds of formulae (I) and (II) as defined in claim 10, vitamin E, and optionally a pharmaceutically, veterinarily or cosmetically acceptable carriers and/or excipients.